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Synthesis, characterization, and in vitro anti-tumor studies of bis imidazolium salts with alkyl chain linkers and naphthylmethyl substituents

Steven R. Crabtree
The University of Akron, src67@zips.uakron.edu

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AUTHOR NAME Steven R. Crabtree[a]

[a]Department of Chemistry, University of Akron, Akron, Ohio 44325, United States.

AUTHOR ADDRESS Department of Chemistry, The University of Akron, Akron, OH 44325-3601, USA. Tel: 330-972-5362 Fax: 330-972-6085 Email: youngs@uakron.edu

Abstract

Bis imidazolium salts, with alkyl chain linkers ranging from methylene to dodecyl, were synthesized with naphthylmethyl substituents at the N^1 and $N^{1'}$ positions for a structure-activity relationship (SAR) study. All compounds were characterized by ^1H and ^{13}C NMR spectroscopy. The cationic portion of **2** as the PF_6 salt, **3**, **4**, and **5** were also characterized by single-crystal X-ray crystallography. Compounds **1-8**, **10**, and **12** were tested for their in vitro anti-cancer activity against four NSCLC cell lines via the MTT assay (NCI–H460, NCI–H1975, HCC827, and A549). Compounds **10** and **12** which contained the decyl and dodecyl chains, respectively, had IC_{50} values of < 1 μM (NCI-H460), < 1 μM (A549), 2 μM (NCI-H1975), and 7 μM (HCC827) and < 1 μM (NCI-H460), < 1 μM (A549), < 1 μM (NCI-H1975), and 4 μM (HCC827), respectively. The results of the MTT assays showed that activity increased as the length of the alkyl linker increased; the bis imidazolium salts with longer chains, compounds **6-8**, **10**, and **12**, had IC_{50} values comparable to cisplatin.The National Cancer Institute's (NCI) Developmental Therapeutics Program (DTP) also tested compounds **1-8**, **10**, and **12** with its 60 human cancer cell line panel in the one-dose assay (10 μM). These results corroborated the SAR findings of our

lab that activity increased with the longer chain alkyls, compounds **6-8**, **10**, and **12**, linking the imidazole rings, with compound **12** being the most active having lethality against all of the NSCLC lines examined and lethality against nearly all cell lines tested.

Keywords: Bis Imidazolium salt; Anti-cancer; Anti-tumor; Lung cancer; SAR study

Abbreviations: NCI – National Cancer Institute; DTP – Developmental Therapeutics Program; IC_{50} – inhibitory concentration 50 %; NSCLC – non-small cell lung cancer; IS29 – 1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide; MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SAR – Structure-Activity Relationship; DNA – Deoxyribonucleic Acid

Introduction

It is estimated that over 595,000 Americans will die of cancer in 2016, and of those deaths over 158,000 will be due to lung cancer; it causes more deaths in both men and women than any other form of cancer, and it accounts for one in four deaths due to cancer. Lung cancer is categorized into small and non-small cell types with the latter making up 83 % of cases.[1] One commonly used class of chemotherapeutics for the treatment of NSCLC is cisplatin and its analogues which are platinum-based drugs. Severe side effects can be expected by patients undergoing platinum-based therapy, e.g. renal dysfunction is experienced by 20 % of patients treated with high doses of cisplatin. Relapse may also occur as cancerous cells become resistant to cisplatin.[2] The five-year survival rate for patients with lung cancer is only 18% which is bleak.[1] New chemotherapeutics are needed to treat NSCLC which do not have as severe side effects. Imidazolium salts (**Figure 1**) may provide a better alternative. Imidazolium salts, including bis imidazolium salts (**Figure 2**), have been synthesized toward many ends, including as ionic liquids and ligands in transition metal chemistry, because of the versatility with which they can be substituted.[3, 4]

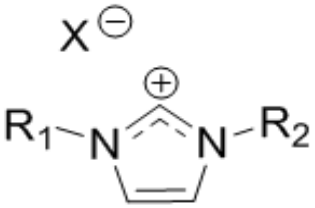


Figure 1. The general structure of an imidazolium salt where X is any singly-charged anion.

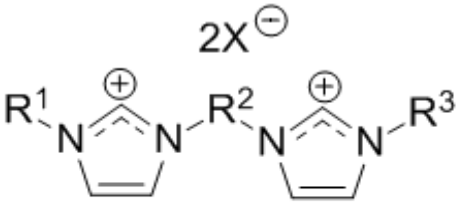


Figure 2. The general structure of a bis imidazolium salt where X can be any singly-charged anion and R^2 can be any linker.

While investigating the anti-tumor ability of silver carbene complexes, our group found that the imidazolium salt precursors to silver NHC's demonstrated high activity against NSCLC lines.[5] A structure-activity relationship (SAR) study was conducted with some earlier imidazolium salts once it was discovered that imidazolium salts with naphthylmethyl groups, as

in the case of 1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (IS29; **Figure 3**), or similar hydrophobic substituents had increased anti-proliferative activity against our NSCLC lines.

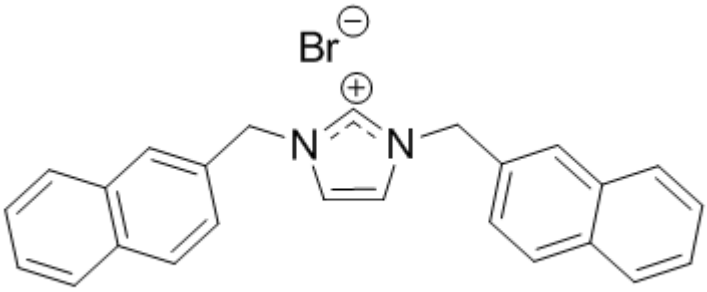


Figure 3. The structure of IS29.

The study kept a naphthylmethyl at the N^1 position and alkyl chains at the N^3 position; salts had increased activity as the length of the alkyl chain increased. The issue with IS29 and salts with highly hydrophobic substituents is that they have poor water solubility which limits their utility.[6]

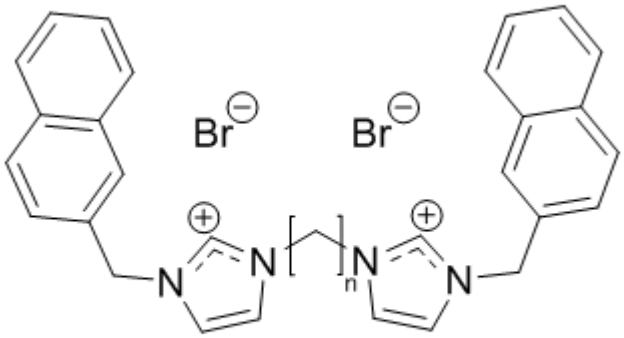


Figure 4. The structure of the synthesized bis imidazolium salts with alkyl chain linkers and naphthylmethyl substituents on the N^1 and $N^{1'}$ atoms.

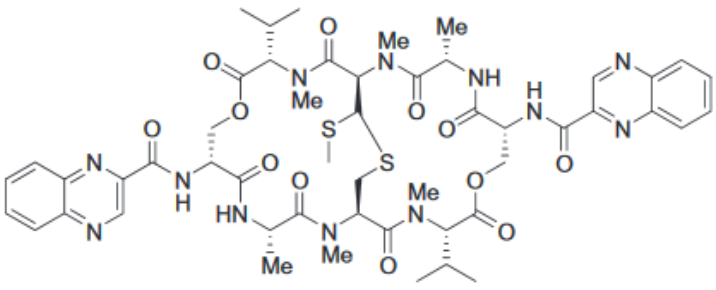


Figure 5. The structure of echinomycin with quinoxaline moieties. Modified from reference 7.

Analogues of IS29 in the form of bis imidazolium salts with alkyl chain linkers (**Figure 4**) will have increased water solubility due to increased in the molecule.

Creating this bridging system may also cause the analogues to have a different modus operandi in halting cell proliferation. These systems would also be analogous to the bis intercalating antibiotic echinomycin (**Figure 5**). Echinomycin is a depsipeptide with two quinoxaline moieties which intercalate double-stranded DNA. Naphthalene is a

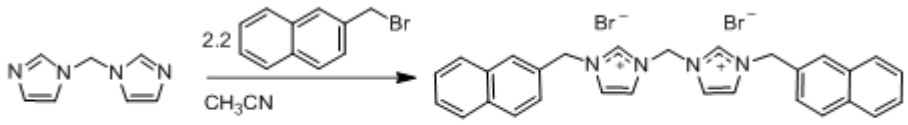
two-membered polycyclic aromatic hydrocarbon, much like quinoxaline, which lends to the notion that this series of compounds could have similar functionality to echinomycin, especially with the flexible alkyl chain also being analogous to the flexible peptide sequence bridging the quinoxaline moieties.[7]

Water solubility and activity will change as the length of the alkyl chain bridging the two imidazole rings changes. Maholtra and Kumar found that imidazolium salts with a methyl group at the N^1 position and alkyl chains at the N^3 position had increased activity as the length of the alkyl chain increased. Salts with alkyl chains with seven or less carbons were not active against the cell lines tested.[8] This agrees with the findings of the Youngs' lab with imidazolium salts with a naphthylmethyl group at the N^1 position and alkyl chains at the N^3 position, and our results show that water solubility drastically declined with increasing alkyl chain. The salt with a naphthylmethyl substituent and an ethyl chain substituent had a solubility of 13.5 mg/mL in water and had IC_{50} values of 26 μM (NCI-H460), > 30 μM (NCI-H1975), and > 30 μM (HCC827) while the salt with a naphthylmethyl substituent and a nonyl chain substituent had a solubility of 0.3 mg/mL in water and had IC_{50} values of 10 μM (NCI-H460), 3 μM (NCI-H1975), and 3 μM (HCC827).[6] Therefore, one can tune the bis imidazolium salt systems by the length of the alkyl chain linker to maximize activity and have sufficient water solubility.

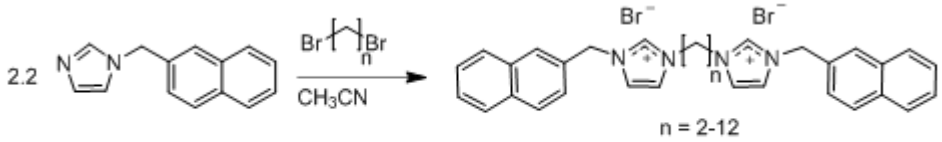
Results and Discussion

Synthesis and characterization

For compound **1** (**Equation 1**), a slight excess of two molar equivalences of 2-(bromomethyl)naphthalene was added to a solution with di(imidazol-1-yl)methane. The solution



Equation 1: Synthesis of compound **1**.



Equation 2: Synthesis of compounds **2-12**.

was refluxed overnight to afford **1** as a white precipitate. **1** was recrystallized in a solution of water and ethanol (1:6). For compounds **2-12** (**Equation 2**), the appropriate dibromo alkane reagent was added to a solution that contained naphthylmethyl imidazole. These solutions were refluxed overnight to produce precipitates for compounds **2-7** and **10**. **3**, **4**, **6**, and **7** were washed with cold acetonitrile to remove unreacted starting materials. **2** was washed with acetone, **5** was recrystallized in a solution of water and ethanol (1:6), and **10** was washed in acetone and diethyl ether. Compounds **8, 9, 11,** and **12** remained soluble even in room temperature acetonitrile. **8** was recrystallized in a solution of water and ethanol (1:5), **9** and **11** were dissolved in water and extracted with diethyl ether, the water was removed under reduced pressure, and methylene chloride was added to the oils to induce precipitation, and **12** was washed in acetone. Compounds **8** and **12** were filtered in open atmosphere, while compounds **9** and **11** were filtered in a glove bag with a nitrogen atmosphere.

All compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and phase transition temperature as none of the compounds formed a liquid at high temperatures and all the compounds eventually degraded. The cationic portion of **2** as the hexafluorophosphate salt and compounds **3**, **4**, and **5** were characterized by single-crystal X-ray crystallography. The evidence for the synthesis of compounds devoid of other organic molecules by ^{1}H NMR was only one resonance detected in the aromatic region where the proton attached to the C2 carbon of the imidazole ring appears ($9.00 < ppm < 10.00$) and a downfield shift of the methylene protons between the imidazole ring and the naphthalene rings from 5.36 for naphthylmethyl imidazole to between 5.50 and 6.00 ppm. In some cases, two resonances were detected with one being much smaller, presumably the mono-cationic species if complete alkylation did not occur, in which case the product was either reacted with additional 2-(bromomethyl)naphthalene or purified via recrystallization. This was the case with compounds **1**, **5**, and **8** which were recrystallized, and compounds **2**, **9**, and **11** which were reacted with additional 2-(bromomethyl)naphthalene.

All resonances in the ^{1}H and ^{13}C were accounted for, but with the ^{1}H NMR spectra and the ^{13}C NMR spectra the aromatic protons and aromatic carbons, respectively, could not feasibly be assigned with surety. The chemical shifts of the methylene protons in the alkyl chains bridging the imidazole rings decreased in ppm the further from the imidazole rings they were due to the deshielding effects of the rings, which was intriguing to examine with the different alkyl chain lengths. The chemical shifts of the carbons in the alkyl chains bridging the imidazole rings also decreased in ppm the further from the imidazole rings there were due to the deshielding effects of the aromatic imidazole rings. With the bis systems, there were some carbons that were equivalent due to symmetry so the integrations were viewed. An integration of 2 was observed for carbons which had at least one hydrogen bonded to it, but carbons that had no hydrogens did

not have correct integrations because their relaxations were presumably too long for accurate integration data to be collected for them. The ^{1}H and ^{13}C NMR spectra collected from compounds **1-12** are included in **Appendix 2**. The ^{1}H and ^{13}C NMR spectra of compounds **1-8**, **10**, and **12** were obtained on a Varian 500 MHz instrument, and the ^{1}H NMR spectra of compounds **9** and **11** were collected on a Varian 300 MHz instrument.

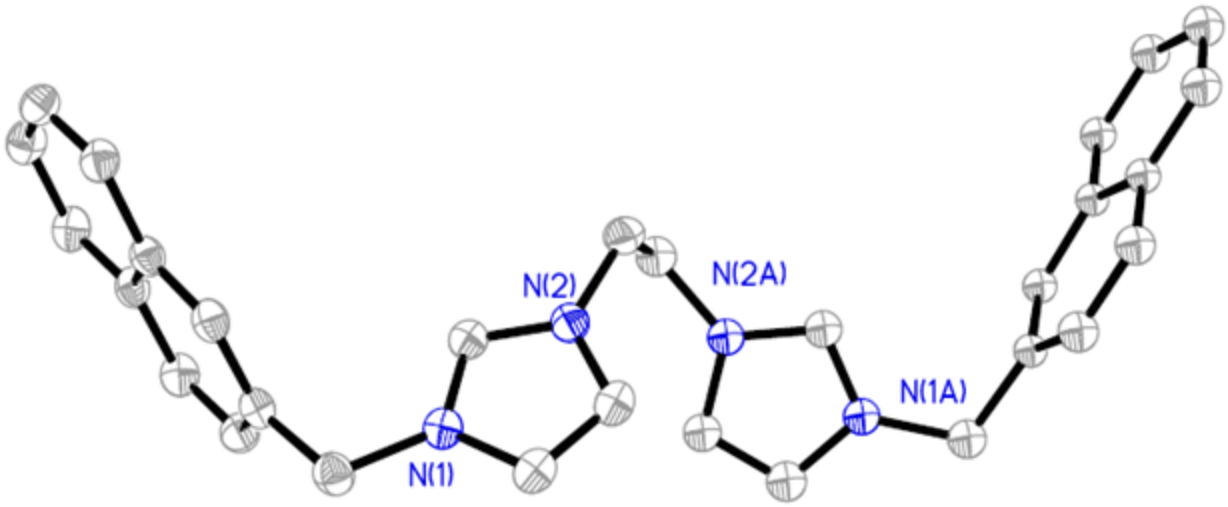


Figure 6. Thermal ellipsoid plot of **2** with the hexafluorophosphate anion with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and carbon labels have been removed for clarity.The figure does not include the PF_6 anions.

The cationic portion of **2** as the hexafluorophosphate salt and compounds **3**, **4**, and **5** were characterized by single-crystal X-ray crystallography. The anion exchange for compound **2** was conducted by dissolving a sample of it in water and adding ammonium hexafluorophosphate. A precipitate formed, the cationic portion of compound **2** with hexafluorophosphate as the anion, which was collected by filtration. A single crystal was collected from the slow evaporation of the compound dissolved in a solution of acetonitrile, chloroform, and 2-propanol (**Figure 6**). The asymmetric unit of **2** was half of the molecule due to the crystallographically imposed mirror plane. The crystals of compounds **3**, **4**, and **5** were grown from the slow evaporation of solvent: compound **3** was grown from a solution of ethyl acetate and tetrahydrofuran (**Figure 7**),

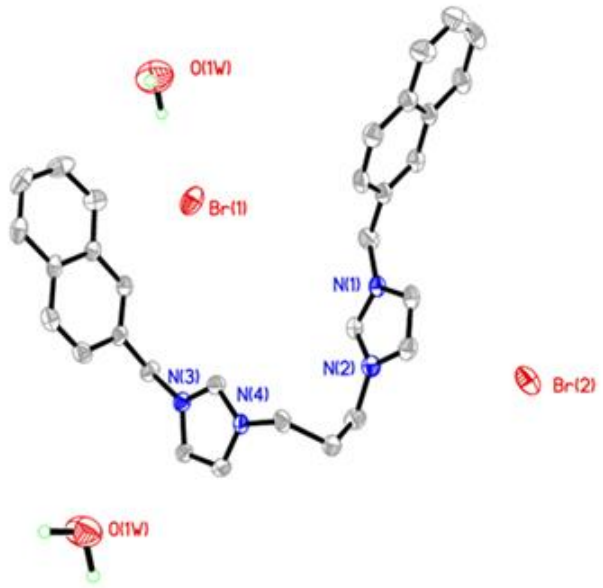


Figure 7. Thermal ellipsoid plot of **3** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and carbon labels have been removed for clarity.

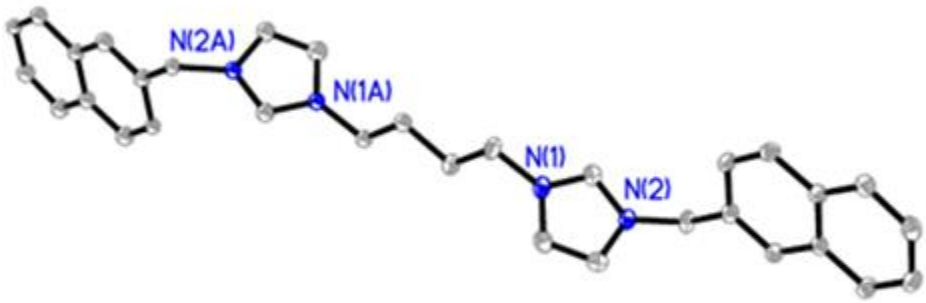


Figure 8. Thermal ellipsoid plot of **4** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and carbon labels have been removed for clarity. The figure does not include the bromide anions.

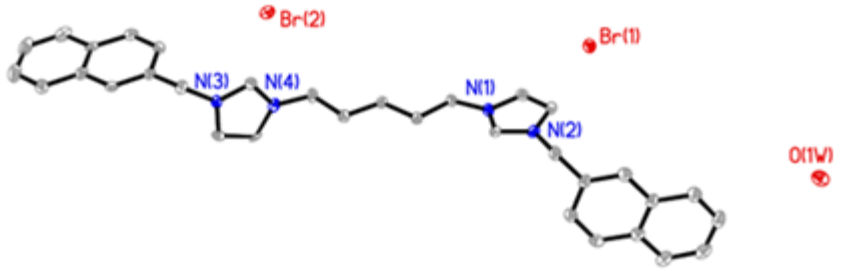


Figure 9. Thermal ellipsoid plot of **5**•(H_2O) with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and carbon labels have been removed for clarity.

compound **4** was grown from methanol (**Figure 8**), and compound **5** was grown from water (**Figure 9**). One molecule of **3** co-crystallized with two water molecules, the asymmetric unit of **4** was half of the molecule due to the crystallographically imposed mirror plane, and one molecule of **5** co-crystallized with one water molecule.

In vitro efficacy studies

The anti-proliferative activities of compounds **1-8**, **10**, and **12** (**Figures 8-11**) were tested

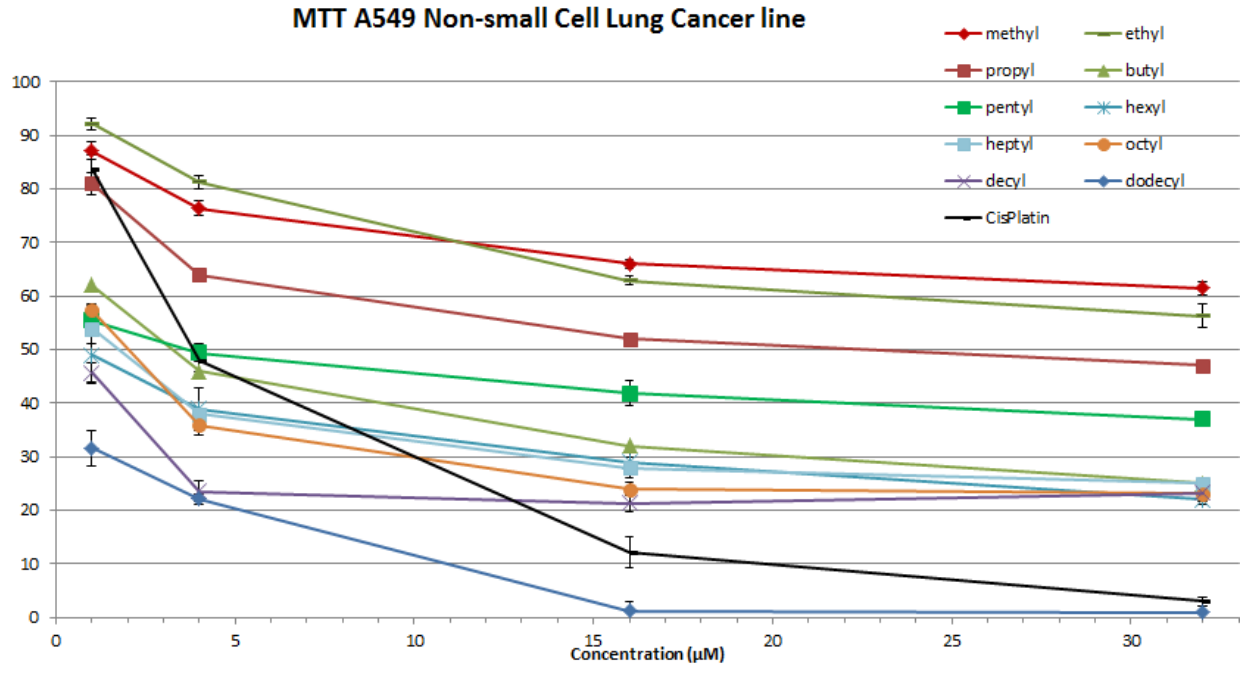


Figure 10. Plot of MTT results from MTT assay of compounds **1-8**, **10**, **12**, and cisplatin against the A549 NSCLC line.

against four non-small cell lung cancer cell lines (NSCLC) (A549, NCI-H460, NCI-H1975, and HCC827). Cells were treated with compounds **1-8**, **10**, **12**, and cisplatin for 72 hours. Cell

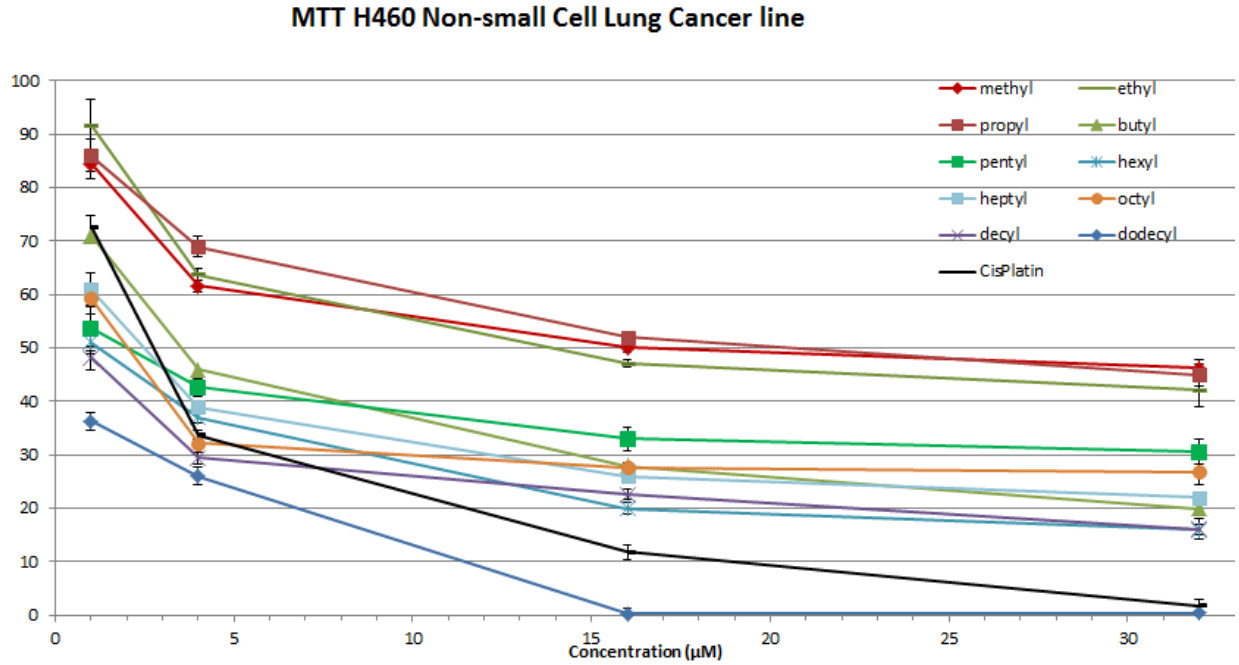


Figure 11. Plot of MTT results from MTT assay of compounds **1-8**, **10**, **12**, and cisplatin against the H460 NSCLC line.

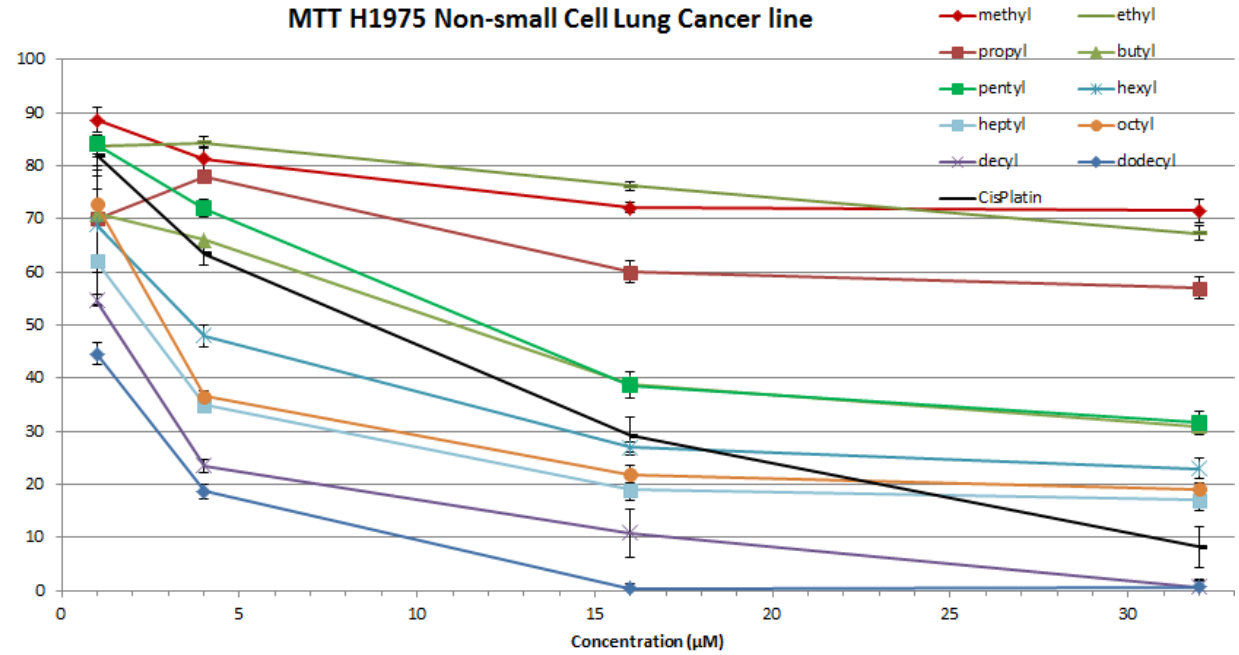


Figure 12. Plot of MTT results from MTT asay of compounds **1-8**, **10**, **12**, and cisplatin against the H1975 NSCLC line.

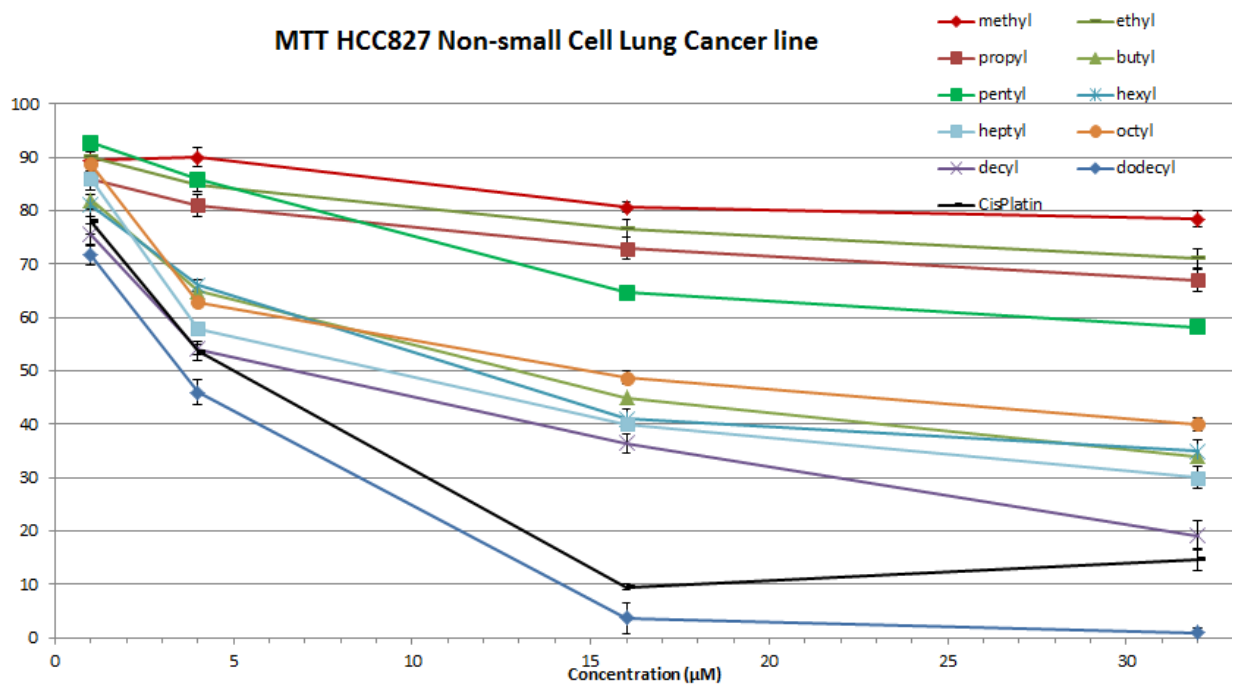


Figure 13. Plot of MTT results from MTT assay of compounds **1-8**, **10**, **12**, and cisplatin against the HCC827 NSCLC line.

viability was determined by the MTT assay after this time period. The anti-proliferative effects of compounds **1-8**, **10**, and **12** were evaluated by their ability to inhibit growth by 50% compared to control cells (IC_{50} value). Solutions of each compound were prepared by dissolving the compound in DMSO and then diluting with water to a final concentration of 1% DMSO in water. The compound solutions were further diluted into growth medium with a maximum DMSO concentration of 0.032% added to the cells.

Compounds **6-8**, **10**, and **12** (**Table 1**) had high activity against all cell lines, but they had less activity against the HCC827 cell line. Compounds **1-5** displayed less activity than the aforementioned compounds with compounds **1-3** having much less activity that was not comparable to cisplatin which had IC_{50} values of 4 µM (H460), 9 µM (H1975), 6 µM (HCC827),

Compound	IC_{50} Values (μM)			
	NCI-H460	NCI-H1975	HCC827	A549
Cisplatin	4	9	6	6
IS29	4	3	4	10
1	16	>30	>30	>30
2	14	>30	>30	>30
3	20	>30	>30	22
4	4	11	13	4
5	2	12	>30	4
6	2	6	12	<1
7	3	3	10	2
8	2	3	15	2
10	<1	2	7	<1
12	<1	<1	4	<1

Table 1. IC_{50} values of cisplatin, IS29, and compounds **1-8**, **10**, and **12** against the NSCLC lines NCI-H460, NCI-H1975, HCC827, and A549.

and 6 μM (A549). IS29 had IC_{50} values of 4 μM (H460), 3 μM (H1975), 4 μM (HCC827), and 10 μM (A549). To compare, compound **12**, which had the highest activity, had IC_{50} values of < 1 μM (H460), < 1 μM (H1975), 4 μM (HCC827), and < 1 μM (A549). The results also show the general trend that as the length of the alkyl chain increases, the anti-proliferative activity is greater. It is well known that hydrophobicity aids in permeating the cell membrane so these results may simply be a result of this.[9] These results are also consistent with that of Malhotra and Kumar. They found that imidazolium salts with a methyl group at the N^1 position and alkyl chains at the N^3 position had increased activity as the length of the alkyl chain did. Salts with alkyl chains with seven or less carbons were not active against the cell lines tested.[8] These results agree with the SAR study of these compounds according to the IC_{50} values for compounds **1-8**, **10**, and **12**. Increasing the length of the alkyl chain bridging the two imidazole rings increases activity, which is the expected result of this study.

The National Cancer Institute's (NCI) Developmental Therapeutics Program (DTP) also tested **1-8**, **10**, and **12** in its 60 human cancer cell line screen using a one-dose assay and agreed

to test **10** and **12** using a five-dose assay. Compounds **1-8** did not have high enough activity to be accepted into the five-dose study. The results from the five-dose assay for **12** have not been obtained. The NCI-60 human cancer cell screen contains nine NSCLC cell lines. Results from the one-dose assay for **1-8**, **10**, and **12** are summarized in **Table 2**. The cells are exposed to one

Compound	Growth Percentage									
	A549/ATCC	EKVX	HOP-62	HOP-92	NCI-H226	NCI-H23	NCI-H322M	MCI-H460	NCI-H522	Average
1	70.78	89.40	84.22	66.99	92.61	88.32	87.60	85.07	50.99	79.55
2	64.07	91.44	85.95	79.18	82.65	88.91	86.40	85.63	63.70	80.88
3	62.32	82.70	67.18	67.47	81.89	77.85	84.40	75.28	56.58	72.85
4	49.31	58.07	54.76	68.41	82.03	44.04	90.05	38.24	43.40	58.70
5	41.68	47.15	57.01	66.04	72.59	45.32	86.17	30.48	38.74	53.91
6	38.64	37.30	55.83	59.93	69.59	30.46	76.24	24.59	33.07	47.29
7	26.68	27.92	32.56	N/A	59.45	14.44	58.62	14.63	5.87	30.02
10	18.55	13.71	-4.86	-23.56	39.46	5.67	34.54	11.00	-72.29	2.47
12	-74.19	-62.61	-58.01	-68.19	-42.06	-80.31	-18.39	-69.88	-74.23	-60.87

Table 2. Growth percentage values for compounds **1-8**, **10**, and **12** against the NSCLC lines in the NCI's DTP 1-dose 60 cell line screen.

concentration, 10 µM, of the compounds in the 60 cell line assay. Results were given as a growth percentage of cells treated with **1-8**, **10**, and **12** compared to growth of control cells. The growth percentages decreased with increasing chain length and eventually were all negative, thereby displaying lethality, at compound **12**. This agrees with the results for IC_{50} values found from our MTT assays run with these compounds that increasing chain length leads to less proliferation. Compound **12** had an average lethality against the nine non-small cell lung cancer cell lines tested of 60.87 % which illustrates the utility of increasing the length of the bridging alkyl chain between the imidazole rings as part of this SAR study.

In the five-dose assay, **10** was exposed to all 60 cell lines at concentrations of 10 nM, 100 nM, 1 µM, 10 µM, and 100 µM. Results were given as concentrations of 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% lethal concentration (LC50) relative to cell growth with no drug added and initial cell count. Results of the five-dose assay for **10** are shown

in **Table 3** and **Figure 14**. The GI50 concentration ranges from the mid nanomolar range (376 nm) to the low µM range (3.55 µM), the TGI concentration ranges from 1.94 µM to 18.4 µM, and the LC50 concentration ranges from 5.95 µM to > 100 µM. These results give further evidence that **10** has potent anti-proliferative activity and merits further studies. Experimental procedures for the one-dose and five-dose assays can be found on the DTP website. However, to

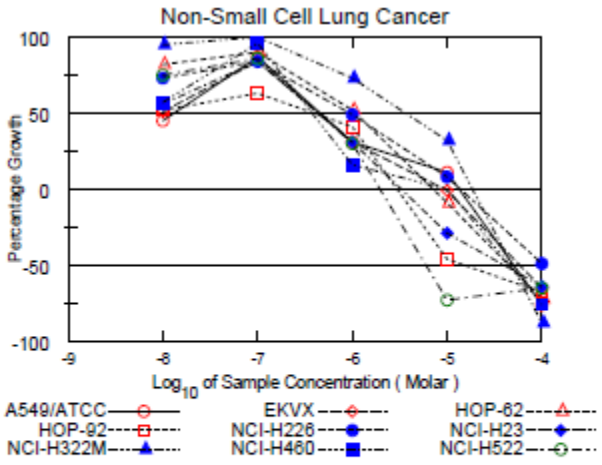


Figure 14. Results from the 5-dose study for compound **10** on the NSCLC lines. Figure taken from NCI results.

Cell Line	Concentration (M)		
	GI50	TGI	LC50
A549/ATCC	N/A	1.33E-05	5.03E-05
EKVX	4.42E-07	9.07E-06	5.84E-05
HOP-62	1.09E-06	7.24E-06	4.45E-05
HOP-92	3.96E-07	2.96E-06	1.52E-05
NCI-H226	9.18E-07	1.36E-05	> 1.00E-4
NCI-H23	4.45E-07	3.35E-06	3.99E-05
NCI-H332M	3.55E-06	1.84E-05	4.81E-05
NCI-H460	3.76E-07	1.01E-05	4.59E-05
NCI-H522	4.36E-07	1.94E-06	5.95E-06

Table 3. The growth inhibition 50 % (GI50), total growth inhibition (TGI), and lethal concentration 50 % concentrations for compound **10** against the NSCLC lines screened by the DTP in its 5-dose studies.

clarify results, growth percentages in the one-dose and five-dose assays performed by the DTP were based on the concentration of proteins at the examined time points. Results from all other cell lines and figures of the tables provided by the DTP are presented in **Appendix 3**.

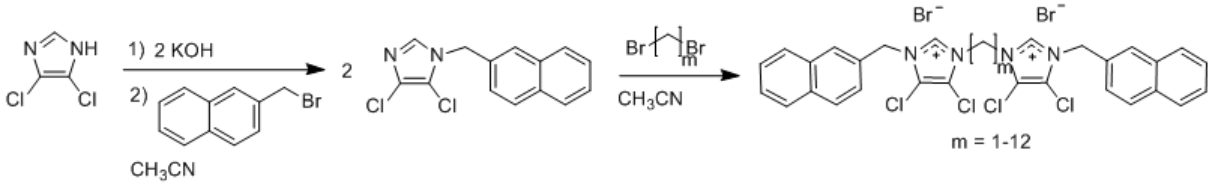
Conclusion

Lipophilic imidazolium salts display anti-proliferative activity against human cancer cell lines. The issue with this is that they are so lipophilic that they are only sparingly soluble in water. The bis imidazolium salts, which are analogous to IS29, display enhanced water solubility when compared to IS29 itself. The water solubility drops off as the length of the alkyl chain

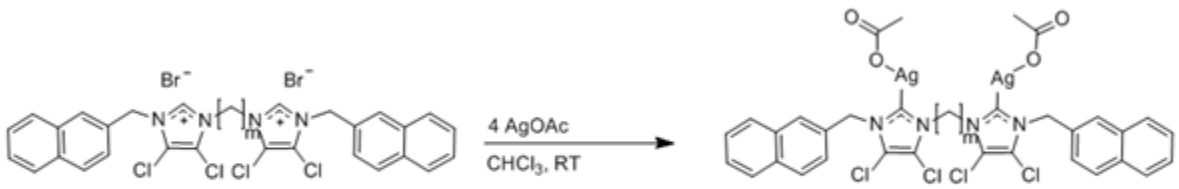
increases, but the anti-proliferative activity increases at the same time, with compound **12** having IC_{50} values greater than both IS29 and cisplatin.

The bis systems with longer chain alkyls, compounds **6-8**, **10**, and **12**, displayed the greatest IC_{50} values as determined from via the MTT assay. These compounds were also accepted into the NCI's DTP 60 cell line 1-dose screen. Both studies affirmed the SAR study that the activity of these compounds increases with increasing alkyl chain length. Compounds **10** and **12** were active enough in the 60 cell line screen to be accepted into the 5-dose screen. Compound **10** had excellent results for the 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% lethal concentration (LC50) against the NSCLC lines studied. This is why further biological studies will be conducted on the dihydro systems in order to determine possible cellular targets.

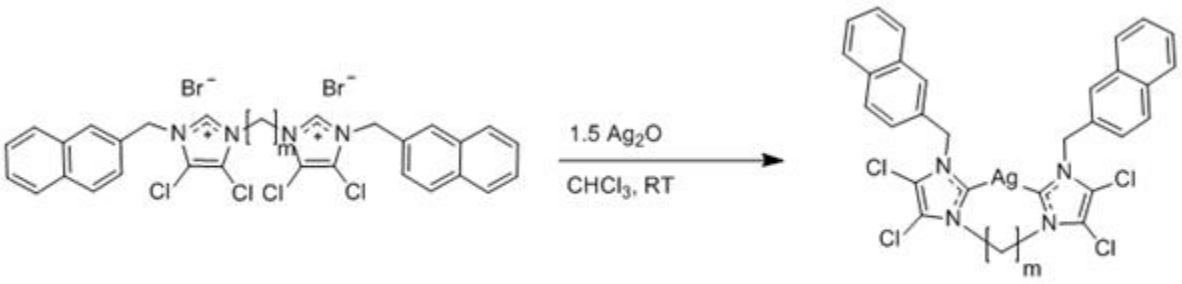
The synthesis and characterization of the 4,5-dichloroimidazole analogues would be the logical next step in the research of bis salts to examine the activity and compare it against the dihydro systems. The proposed synthesis of this series of compounds is similar to the synthesis of compounds **2-12** (**Scheme 1**). These systems would also have the ability to coordinate to silver centers as NHC ligands for use as antibiotics (**Equations 3** and **4**). These compounds will



Scheme 1. Route proposed for synthesis of 4,5-dichloro bis imidazolium salts with alkyl chain linkers and naphthylmethyl substituents.



Equation 3. The synthesis of SCC silver acetate complexes with bis imidazolium salts.



Equation 4. The synthesis of doubly-coordinate, bridging SCC's with bis imidazolium salts.

have cytotoxicity against eukaryotic cells as well as microbes once they are coordinated to silver centers. This would lend these compounds to being excellent candidates for dual purpose drugs, such as bladder exfoliants for treatment of recurrent urinary tract infections.[10]

Experimental Section

General Procedures

All reactions were conducted under aerobic conditions except where indicated. 2-(Bromomethyl)naphthalene was purchased from Waterstone Technologies. 1,2-dibromoethane was purchased from Baker, 1,3-dibromopropane, 1,4-dibromobutane, 1,6-dibromohexane, and 1,8-dibromooctane were purchased from Acros Organics, 1,5-dibromopentane was purchased from Alfa Aesar, 1,7-dibromoheptane and 1,10-dibromodecane were purchased from TCI, 1,11-dibromoundecane was purchased from Aldrich, and 1,12-dibromododecane was purchased from Avocado. Imidazole was purchased from Acros Organics. Naphthylmethyl imidazole was synthesized according to literature procedures.[11] Di(imidazol-1-yl)methane (0.50 g, 3.4 mmol) was dissolved in hot chloroform and filtered to remove yellow particulates. The filtrate was

cooled to room temperature to produce crystals of di(1H-imidazol-1-yl)methane which was filtered. All solvents were purchased from Fisher Scientific. All reagents were used as received without further purification. ^{1}H and ^{13}C NMR spectra were obtained on a Varian 500 MHz instrument with all spectra referenced to residual deuterated solvent for compounds **1**-**8**, **10**, and **12** (DMSO-d6: ^{1}H NMR: 2.50 ppm, ^{13}C NMR: 39.5 ppm). ^{1}H and ^{13}C NMR spectra were obtained on a Varian 300 MHz instrument with all spectra referenced to residual deuterated solvent for compounds **9** and **11** (DMSO-d6: 1H NMR: 2.50 ppm, 13C NMR: 39.5 ppm).

Single-Crystal X-ray Crystallography Procedures

Crystals of the compounds were coated in paratone oil, mounted on a CryoLoop and placed on the goniometer head under a stream of nitrogen (100 K). The data sets were collected on a Bruker Kappa APEX II Duo CCD system equipped with a Mo ImuS (λ = 0.71073 Å) source and a Cu ImuS micro-focus source equipped with QUAZAR optics (λ = 1.54178 Å). The unit cells were determined by using reflections from three different orientations. Data sets were integrated using SAINT.[12] An empirical absorption correction and other corrections were applied to the data sets using multi-scan SADABS. Structure solution, refinement, and modeling were accomplished by using the Bruker SHELXTL package.[13] The structures were determined by full-matrix least-squares refinement of F^2 and the selection of the appropriate atoms from the generated difference map. Hydrogen atom positions were calculated and U_{iso}(H) values were fixed according to a riding model.

The human NSCLC cell lines NCI-H1975 and HCC827 were generously provided by Dr. Lindner from the Cleveland Clinic. The human NSCLC cell lines NCI-H460 and NCI-A549 were purchased from ATCC (Manassas, VA, USA). All cell lines were grown at 37 °C with 5%

CO_2 in RPMI 1640 medium supplemented with 10% fetal bovine serum and passed every 2-3 days.

MTT assay

Cells were grown to confluence and plated in 96-well plates at 5,000-7,000 cells per well, depending on the cell line. Cells were incubated for 24 h prior to adding the compounds. All compounds were dissolved in a 1% DMSO solution and diluted in fresh medium to the desired concentrations of 1, 4, 16, and 32 μM. Compounds were added (6 replicates each) and cells were incubated for 72 h at which time the MTT assay protocol was followed. MTT reagent (10 μL) was added to each well and cells were incubated for 3-4 h, again depending on the cell line. Growth medium was removed by aspiration and DMSO (100 μL) was added to each well. Plates were incubated for 15 min. The optical density was read at 540 nm on a Biotek Epoch plate reader.

Synthesis of 1-(naphthalen-2-ylmethyl)-3-((3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)methyl)-imidazolium bromide (1)

Di(1H-imidazol-1-yl)methane (0.12 g, 0.8 mmol) was dissolved in acetonitrile (7 mL) and 2-(bromomethyl)naphthalene (0.40 g, 1.8 mmol) was added. The reaction was heated and stirred at 70 °C overnight. The volatile components were removed under reduced pressure, and the white solid was washed with acetone (25 mL) and chloroform (15 mL), subsequently. The product was recrystallized in a solution of water and ethanol (1:6). After filtration, the crystals were ground to a fine powder by mortar and pestle and residual volatile components were removed under reduced pressure to purify the white solid (0.42 g, 87 % yield). Found C, 58.51; H, 4.28; N, 9.45%. Calculated for $C_{29}H_{26}N_4Br_2$: C, 59.0; H, 4.4; N, 9.5%. Phase Transition, 283 – 285°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.70 (2H, s, Ar), 8.13-7.93 (12H, m, Ar), 7.59-7.57

(6H, m, Ar), 6.72 (2H, s, CH_2), 5.68 (4H, s, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 137.8 (NCN), 132.8 (Ar), 132.6 (Ar), 131.5 (Ar), 128.7 (Ar), 128.0 (Ar), 127.8 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.9 (Ar), 123.3 (Ar), 122.5 (Ar), 58.3 (CH_2), 52.5 (CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(2-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)ethyl)-imidazolium bromide (2)

1,2-Dibromoethane (56 μL, 0.7 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.30 g, 1.4 mmol) were heated in acetonitrile (5 mL) overnight. The volatile components were removed under reduced pressure, and the white solid was triturated with acetone and filtered. Residual volatile components were removed under reduced pressure to yield a white solid (0.06 g, 16 % yield). Found C, 60.67; H, 4.21; N, 9.37%. Calculated for **1**: C, 59.6; H, 4.7; N, 9.3%. Phase Transition, 285°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.32 (2H, s, Ar), 7.98-7.91 (8H, m, Ar), 7.86 (2H, dd, Ar), 7.73 (2H, dd, Ar), 7.58-7.57 (4H, m, Ar), 7.48 (1H, d, Ar), 7.46 (1H, d, Ar), 5.57 (4H, s, CH_2), 4.75 (4H, t, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.8 (NCN), 132.7 (Ar), 132.6 (Ar), 131.8 (Ar), 128.7 (Ar), 127.8 (Ar), 127.63 (Ar), 127.60 (Ar), 126.8 (Ar), 126.7 (Ar), 125.6 (Ar), 122.94 (Ar), 122.86 (Ar), 52.2 (CH_2), 48.5 (CH_2).

Crystal data for 1,1'-methylenebis(3-(naphthalen-2-ylmethyl)-imidazolium) hexafluorophosphate(V): $C_{30}H_{28}F_{12}N_4P_2$, M = 734.50, monoclinic, a = 35.130(2) Å, b = 6.9080(4) Å, c = 12.5665(8) Å, β = 101.406(3)°, V = 2989.4(3) Å^3, T = 100(2) K, space group C2/c, Z = 4, 11425 reflections measured, 3039 independent reflections (R_{int} = 0.0413). The final R_1 values were 0.0477 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1154 ($I > 2\sigma(I)$). The final R_1 values were 0.0713 (all data). The final $wR(F^2)$ values were 0.1308 (all data).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(3-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)propyl)-imidazolium bromide (3)

1,3-Dibromopropane (66 μL, 0.7 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.30 g, 1.4 mmol) were heated in acetonitrile (5 mL) overnight. The clear solution was cooled (-20 °C) to induce precipitation. The white power was filtered and washed with cold (-20 °C) acetonitrile (25 mL). A fine, white powder was collected (0.34 g, 84 % yield). Found C, 55.69; H, 4.94; N, 8.61%. Calculated for **2**: C, 60.2; H, 4.9; N, 9.1%. Phase Transition, 145 – 147°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.49 (2H, s, Ar), 8.01-7.89 (12H, m, Ar), 7.59-7.55 (6H, m, Ar), 5.62 (4H, s, CH_2), 4.30 (4H, t, CH_2), 2.46 (2H, p, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.5 (NCN), 132.7 (Ar), 132.6 (Ar), 132.0 (Ar), 128.7 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.7 (Ar), 126.6 (Ar), 125.8 (Ar), 122.7 (Ar), 122.6 (Ar), 52.1 (CH_2), 46.0 (CH_2), 29.3 (CH_2).

Crystal data for **3**: $C_{31}H_{30}N_4Br_2$, M = 636.42, monoclinic, a = 21.4487(8) Å, b = 12.5771(4) Å, c = 10.6265(4) Å, β = 91.5181(17)°, V = 2865.62(18) Å^3, T = 100(2) K, space group P2$_1$/c, Z = 4, 51189 reflections measured, 5811 independent reflections (R_{int} = 0.0435). The final R_1 values were 0.0335 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0784 ($I > 2\sigma(I)$). The final R_1 values were 0.0520 (all data). The final $wR(F^2)$ values were 0.0861 (all data).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(4-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)butyl)-imidazolium bromide (4)

1,4-Dibromobutane (78 μL, 0.7 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.30 g, 1.4 mmol) were heated in acetonitrile (5 mL) overnight. A white precipitate formed, and the mixture was cooled (-20 °C) to induce further precipitation. The white power was filtered and washed with cold (-20 °C) acetonitrile (25 mL). A fine, white powder was collected (0.32 g, 76 % yield). Found C, 57.62; H, 5.13; N, 8.33%. Calculated for **4**: C, 60.8; H, 5.1; N, 8.9%. Phase Transition, 223 – 225°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.37 (2H, s, Ar), 7.98-7.83 (12H,

m, Ar), 7.58-7.52 (6H, m, Ar), 5.60 (4H, s, CH_2), 4.23 (4H, t, CH_2), 1.81 (4H, t, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.3 (NCN), 132.68 (Ar), 132.65 (Ar), 132.1 (Ar), 128.7 (Ar), 127.8 (Ar), 127.63 (Ar), 127.57 (Ar), 126.74 (Ar), 126.71 (Ar), 125.7 (Ar), 122.71 (Ar), 122.69 (Ar), 52.1 (CH_2), 48.2 (CH_2), 26.0 (CH_2).

Crystal data for **4**: $C_{32}H_{36}N_4O_2Br_2$, M = 668.47, monoclinic, a = 11.8237(4) Å, b = 11.7179(3) Å, c = 10.6439(3) Å, β = 99.8580(10)°, V = 1452.93(7) Å^3, T = 100(2) K, space group $P2_1/c$, Z = 2, 15219 reflections measured, 2950 independent reflections (R_{int} = 0.0330). The final R_1 values were 0.0307 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0801 ($I > 2\sigma(I)$). The final R_1 values were 0.0377 (all data). The final $wR(F^2)$ values were 0.0849 (all data).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(5-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)pentyl)-imidazolium bromide (5)

1,5-Dibromopentane (178 μL, 1.3 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.60 g, 2.9 mmol) were heated in acetonitrile (4 mL) overnight. The volatile components were removed under reduced pressure, and the white solid was triturated and filtered with cold (-20 °C) acetone (25 mL). The white powder was recrystallized in a solution of water and ethanol (1:6). The crystals were filtered and washed with acetone (15 mL). The volatile components were removed under reduced pressure to yield a white powder (0.44 g, 52 % yield). Phase Transition, 226 – 228°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.49 (2H, s, Ar), 8.00-7.87 (12H, m, Ar), 7.58-7.54 (6H, m, Ar), 5.63 (4H, s, CH_2), 4.21 (4H, t, CH_2), 1.85 (4H, tt, CH_2), 1.25 (2H, p, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.1 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 126.70 (Ar), 126.67 (Ar), 125.7 (Ar), 122.7 (Ar), 122.6 (Ar), 52.1 (CH_2), 48.5 (CH_2), 28.5 (CH_2), 22.0 (CH_2).

Crystal data for **5**: $C_{33}H_{36}N_4Br_2O$, M = 664.48, monoclinic, a = 10.7412(9) Å, b = 23.605(3) Å, c = 11.5324(13) Å, β = 90.046(4)°, V = 2924.0(5) Å^3, T = 100(2) K, space group $P2_1/n$, Z = 4, 38687 reflections measured, 5562 independent reflections (R_{int} = 0.0694). The final R_1 values were 0.0334 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0735 ($I > 2\sigma(I)$). The final R_1 values were 0.0467 (all data). The final $wR(F^2)$ values were 0.0786 (all data).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(6-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)hexyl)-imidazolium bromide (6)

1,6-Dibromohexane (135 μL, 0.9 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.40 g, 1.9 mmol) were heated in acetonitrile (7 mL) overnight. A white precipitate formed, and the reaction mixture was cooled (-20 °C) to induce further precipitation. The white precipitate was filtered and washed with cold (-20 °C) acetonitrile (30 mL) (0.44 g, 77 % yield). Found C, 61.62; H, 5.31; N, 8.42%. Calculated for **6**: C, 61.8; H, 5.5; N, 8.5%. Phase Transition, 233 – 234°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.57 (2H, s, Ar), 8.02-7.89 (12H, m, Ar), 7.59-7.54 (6H, m, Ar), 5.66 (4H, s, CH_2), 4.20 (4H, t, CH_2), 1.81 (4H, tt, CH_2), 1.27 (4H, t, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.8 (Ar), 127.58 (Ar), 127.56 (Ar), 126.7 (Ar), 126.6 (Ar), 125.7 (Ar), 122.7 (Ar), 122.5 (Ar), 52.0 (CH_2), 48.7 (CH_2), 28.9 (CH_2), 24.7 (CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(7-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)heptyl)-imidazolium bromide (7)

1,7-Dibromoheptane (149 μL, 0.9 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.40 g, 1.9 mmol) were heated in acetonitrile (7 mL) overnight. The clear solution was cooled (-20 °C) and the white precipitate which formed was washed with cold (-20 °C) acetonitrile (25 mL). The white powder was dried via aspirator filtration (0.38 g, 64 % yield). The solubility of the

product in deionized water is 19.5 mg/mL. Found C, 60.94; H, 5.67; N, 8.06%. Calculated for **7**: C, 62.3; H, 5.7; N, 8.3%. Phase Transition, 198 – 200°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.53 (2H, s, Ar), 8.00-7.88 (12H, m, Ar), 7.58-7.54 (6H, m, Ar), 5.64 (4H, s, CH_2), 4.19 (4H, t, CH_2), 1.79 (4H, tt, CH_2), 1.29 (2H, tt, CH_2), 1.22 (2H, p, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.8 (Ar), 127.60 (Ar), 127.55 (Ar), 126.7 (Ar), 126.6 (Ar), 125.6 (Ar), 122.7 (Ar), 122.6 (Ar), 52.0 (CH_2), 48.8 (CH_2), 29.0 (CH_2), 27.5 (CH_2), 25.2 (CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(8-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)octyl)-imidazolium bromide (8)

1,8-Dibromooctane (161 μL, 0.9 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.40 g, 1.9 mmol) were heated in acetonitrile (3 mL) overnight. The volatile components were removed via rotary evaporation under reduced pressure. The off-white powder was washed and filtered with cold (-20 °C) acetone (25 mL). The powder was recrystallized in an ethanol and water solution (5:1). The crystals were washed and filtered with cold (-20 °C) acetone (15 mL). The crystals were crushed to an off-white powder which was dried under reduced pressure (0.50 g, 83 % yield). Found C, 59.58; H, 5.57; N, 7.65%. Calculated for **8**: C, 62.8; H, 5.9; N, 8.1%. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.46 (2H, s, Ar), 7.99-7.86 (12H, m, Ar), 7.57-7.54 (6H, m, Ar), 5.63 (4H, s, CH_2), 4.18 (4H, t, CH_2), 1.78 (4H, tt, CH_2), 1.24-1.20 (8H, m, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 126.70 (Ar), 126.68 (Ar), 125.6 (Ar), 122.7 (Ar), 122.6 (Ar), 52.1 (CH_2), 48.9 (CH_2), 29.1 (CH_2), 28.1 (CH_2), 25.3 (CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(9-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)nonyl)-imidazolium bromide (9)

1,9-Dibromoundecane (293 μL, 1.4 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.600 g, 2.9 mmol) were heated in acetonitrile (4 mL) overnight. The volatile components from the reaction were removed under reduced pressure. The resulting viscous, white oil was dissolved in deionized water (300 mL) and extracted twice with diethyl ether (100 mL). The water layer was collected and the water was removed under reduced pressure. The viscous, transparent, and tan oil was triturated with methylene chloride (100 mL) which induced precipitation of a tan solid. The mixture was filtered in a glove bag with a nitrogen environment. The tan solid was collected and stored at ambient conditions in open atmosphere (0.522 g, 53 % yield). Calculated for **9**: C, 63.7; H, 6.2; N, 7.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.43 (2H, s, Ar), 7.98-7.84 (12H, m, Ar), 7.59-7.53 (6H, m, Ar), 5.64 (4H, s, CH_2), 4.18 (4H, t, CH_2), 1.78 (4H, tt, CH_2), 1.23-1.20 (10H, m, CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(10-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)decyl)-imidazolium bromide (10)

1,10-Dibromodecane (0.26 g, 0.9 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.40 g, 1.9 mmol) were heated in acetonitrile (3 mL) overnight. The reaction mixture was cooled (-20 °C) because a white precipitate formed at room temperature. The chilled mixture was filtered and the white solid was washed with acetone (25 mL). The white solid was subsequently washed with diethyl ether (25 mL) three times. The white solid was dried under reduced pressure (0.31 g, 49 % yield). Found C, 62.88; H, 6.26; N, 7.61%. Calculated for **10**: C, 63.7; H, 6.2; N, 7.8%. Phase Transition, 66°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.43 (2H, s, Ar), 7.99-7.85 (12H, m, Ar), 7.59-7.53 (6H, m, Ar), 5.62 (4H, s, CH_2), 4.18 (4H, t, CH_2), 1.78 (4H, tt, CH_2), 1.22-1.20

(12H, m, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.8 (Ar), 127.60 (Ar), 127.55 (Ar), 126.7 (Ar), 126.6 (Ar), 125.6 (Ar), 122.7 (Ar), 122.6 (Ar), 52.0 (CH_2), 48.8 (CH_2), 29.0 (CH_2), 27.5 (CH_2), 25.2 (CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(11-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)undecyl)-imidazolium bromide (11)

1,11-Dibromoundecane (340 μL, 1.4 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.601 g, 2.9 mmol) were heated in acetonitrile (4 mL) overnight. The volatile components from the reaction were removed under reduced pressure. The resulting viscous, white oil was dissolved in deionized water (300 mL) and extracted twice with diethyl ether (100 mL). The water layer was collected and the water was removed under reduced pressure. The viscous, clear oil was triturated with methylene chloride (100 mL) which induced precipitation of a white solid. The mixture was filtered in a glove bag with a nitrogen environment. The solid was taken out of the bag which caused it to phase transition to a clear oil again. The oil was dissolved in chloroform (40 mL) and evaporated at ambient conditions in open atmosphere. A white, crystalline solid was collected (0.182 g, 18 % yield). Calculated for **11**: C, 64.1; H, 6.4; N, 7.7%. ^1H NMR (300 MHz, DMSO-d_6) δ = 9.41 (2H, s, Ar), 7.99-7.87 (12H, m, Ar), 7.58-7.53 (6H, m, Ar), 5.63 (4H, s, CH_2), 4.18 (4H, t, CH_2), 1.78 (4H, tt, CH_2), 1.23-1.19 (14H, m, CH_2).

Synthesis of 1-(naphthalen-2-ylmethyl)-3-(12-(3-(naphthalen-2-ylmethyl)-imidazolium-1-yl)dodecyl)-imidazolium bromide (12)

1,12-Dibromododdecane (0.29 g, 0.9 mmol) and 1-(naphthalen-2-ylmethyl)-imidazole (0.40 g, 1.9 mmol) were heated in acetonitrile (3 mL) overnight. The volatile components were removed under reduced pressure and the off-white solid which formed was washed with acetone (25 mL). The solid became a highly-viscous gel on the filter paper. After a week of being

undisturbed in a fume hood, the hardened, tan product was ground and collected (0.31 g, 48 % yield). The solubility of the product in deionized water is 2.6 mg/mL. Found C, 63.83; H, 6.45; N, 7.40%. Calculated for **12**: C, 64.5; H, 6.5; N, 7.5%. Phase Transition, 137 – 138°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.46 (2H, s, Ar), 7.98-7.86 (12H, m, Ar), 7.58-7.54 (6H, m, Ar), 5.63 (4H, s, CH_2), 4.19 (4H, t, CH_2), 1.79 (4H, tt, CH_2), 1.23-1.19 (12H, m, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.7 (Ar), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 126.68 (Ar), 126.65 (Ar), 125.6 (Ar), 122.7 (Ar), 122.6 (Ar), 52.0 (CH_2), 48.9 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 28.3 (CH_2), 25.5 (CH_2).

Supporting Information. Safety consideration of this project, the ^{1}H and ^{13}C NMR for compounds **1-12**, and the NCI 1- and 5-dose data sheets are included in the supporting information as **Appendices 1**, **2**, and **3**, respectively.

Acknowledgments

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Appendix 1: Safety Considerations

There were various, serious safety considerations that were taken into account as this project progressed. A basic precaution was that gloves and safety glasses were worn at all times in the lab to prevent exposure to various chemicals in the lab. When using a base bath to clean glassware, a lab coat and acid gloves were worn to prevent exposure to the alcoholic hydroxide solution when placing items into or taking items out of the base bath. In a few instances, aqua regia was used to clean NMR tubes and other pieces of glassware that the base bath did not clean. A lab coat and acid gloves were worn when preparing aqua regia solutions, and the solutions were always carefully prepared in a fume hood with sodium bicarbonate spread around the container holding the solution. Aqua regia was neutralized with sodium bicarbonate to a neutral pH, and the resulting solution was disposed of in an aqueous waste container.

The greatest safety hazard were the chemicals utilized in the synthesis of these compounds and the compounds themselves. The brominated reagents, the dibromo alkyl reagents and 2-(bromomethyl)naphthalene, are carcinogenic irritants. Care was taken to not be exposed to fumes from the reagent containers, and the reagents were carefully transferred to the analytical balance and from the balance to the reaction flasks. The most dangerous chemicals were the products themselves because the results obtained from the MTT assays and from the NCI results show that they are highly cytotoxic. Similar imidazolium salts synthesized by this group were found to be fatal to mice at a concentration of 30 mg/kg body weight. An average adult male weights 70 kg, so it would take roughly 2 g of the more toxic compounds to kill someone. This shows that even slight inhalation over time of these compounds is extremely hazardous. This was a real concern because many of the compounds formed fine powders with a great deal of electrostatic interactions which made transferring them to vials rather difficult. The products had

to be transferred using a spatula in most cases, and even then they stuck to the weigh paper and had a tendency to become airborne in small amounts. This is why these transfers were completed in a fume hood with the sash down as far as possible while still allowing mobility of arms inside of it.

Appendix 2: ^{1}H and ^{13}C NMR Spectra

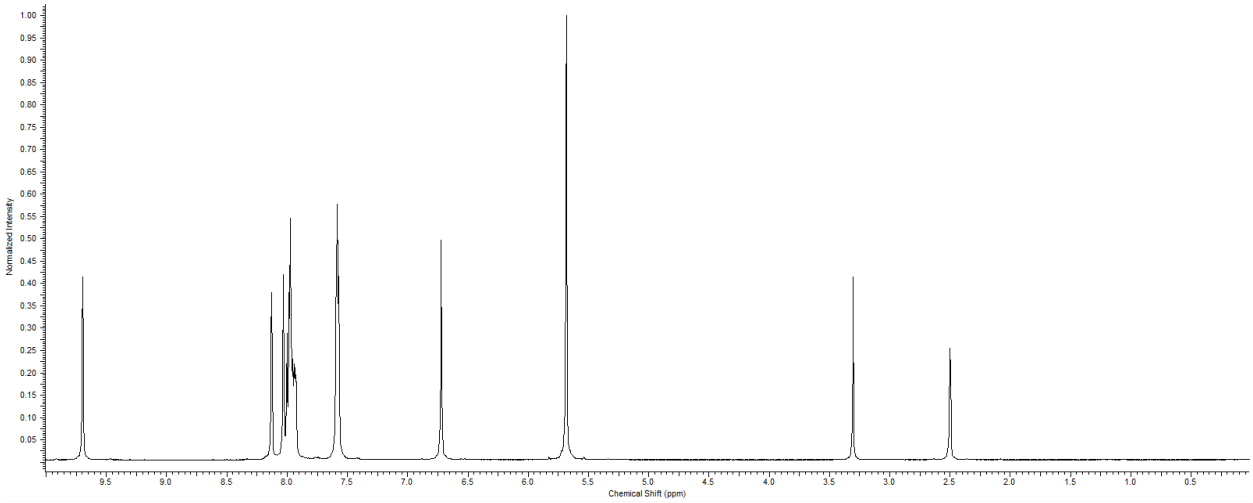


Figure A2.1. ^{1}H NMR spectrum of **1**.

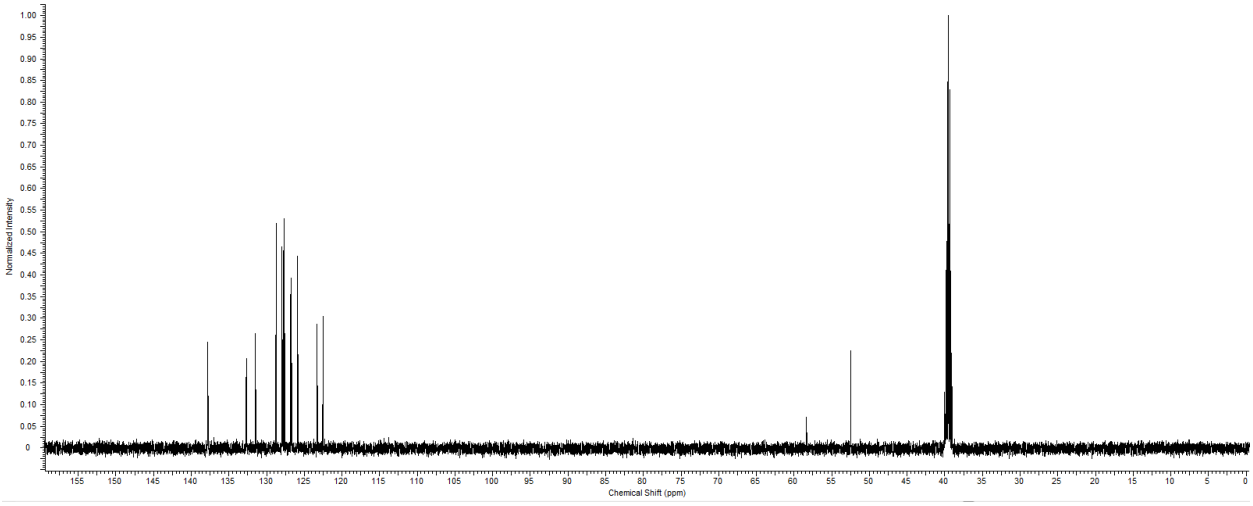


Figure A2.2. ^{13}C NMR spectrum of **1**.

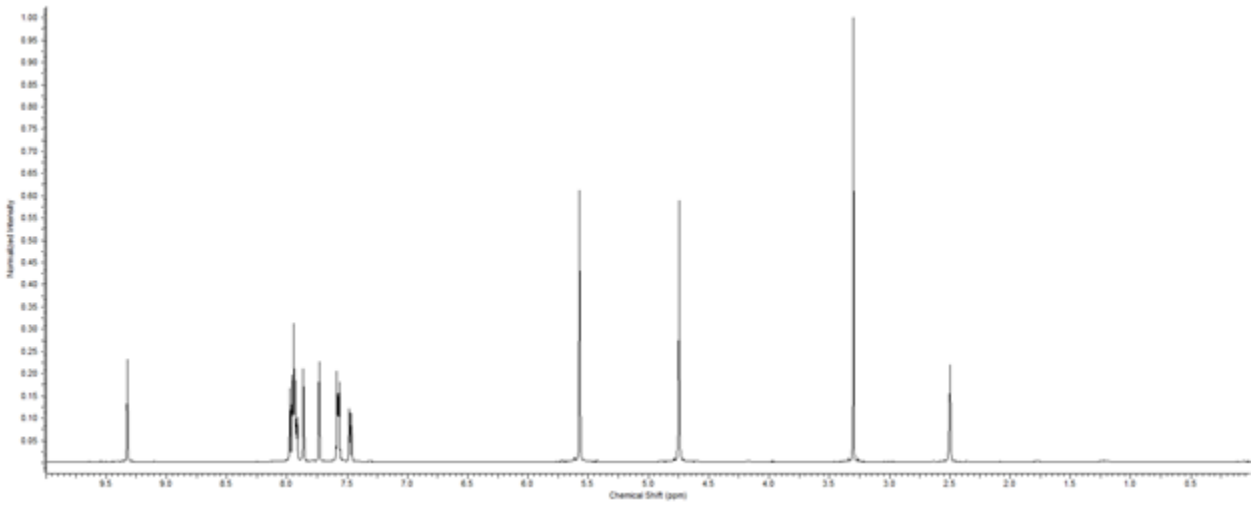


Figure A2.3. ^{1}H NMR spectrum of **2**.

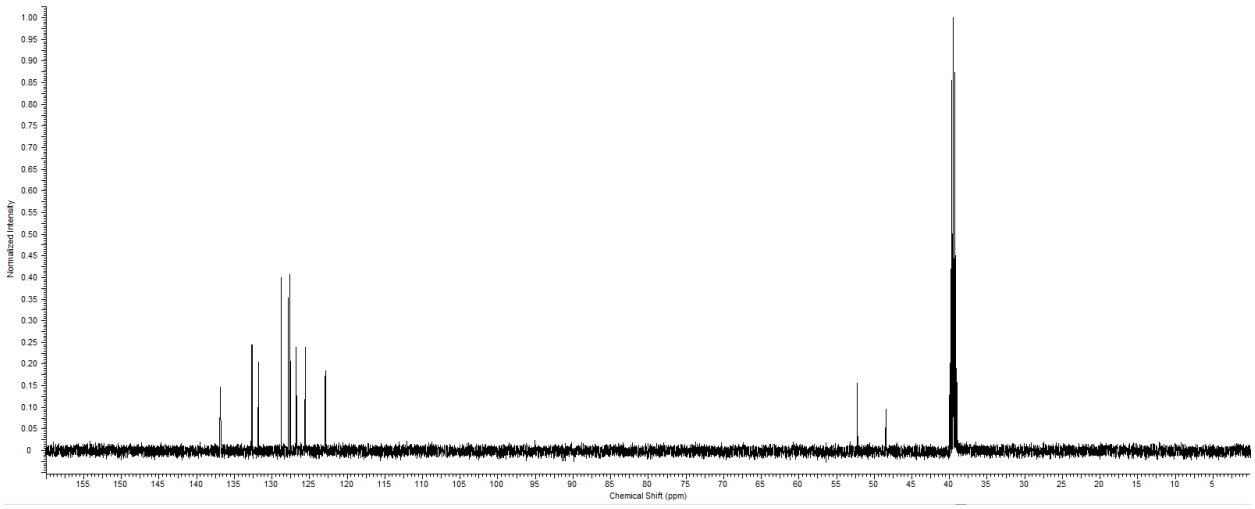


Figure A2.4. ^{13}C NMR spectrum of **2**.

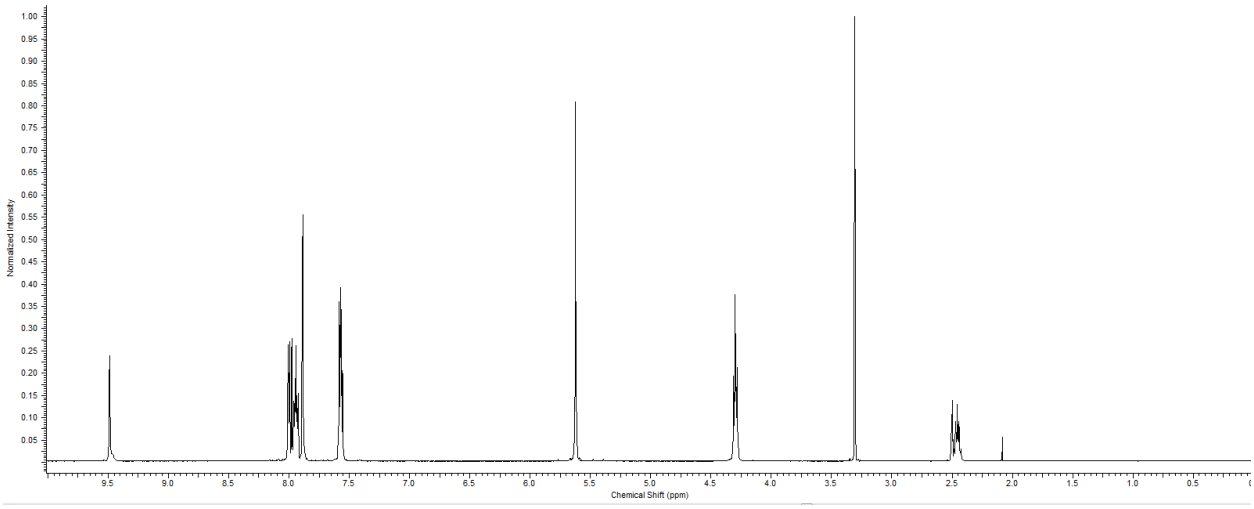


Figure A2.5. ^{1}H NMR spectrum of **3**.

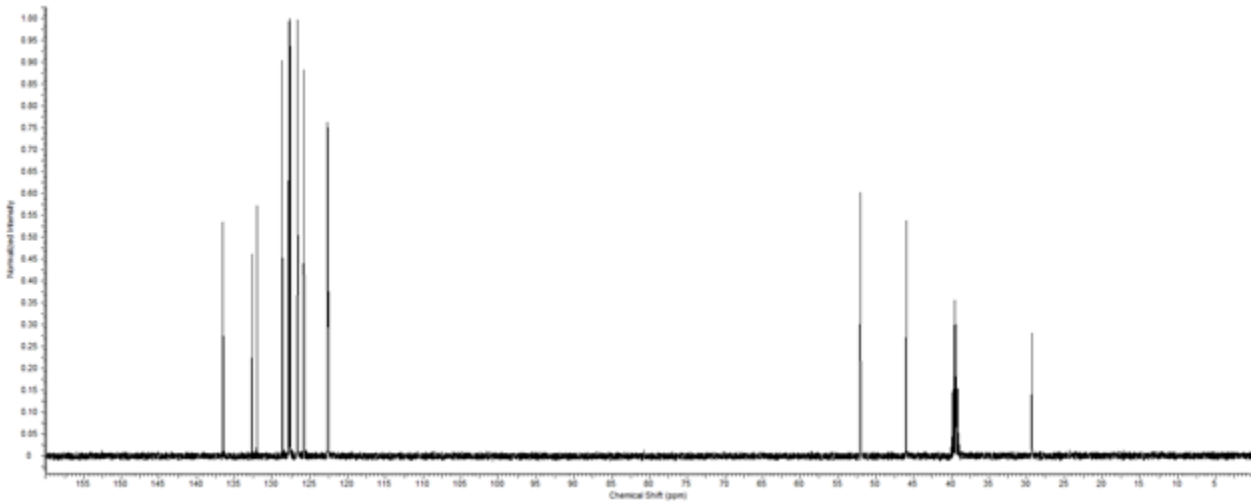


Figure A2.6. ^{13}C NMR spectrum of **3**.

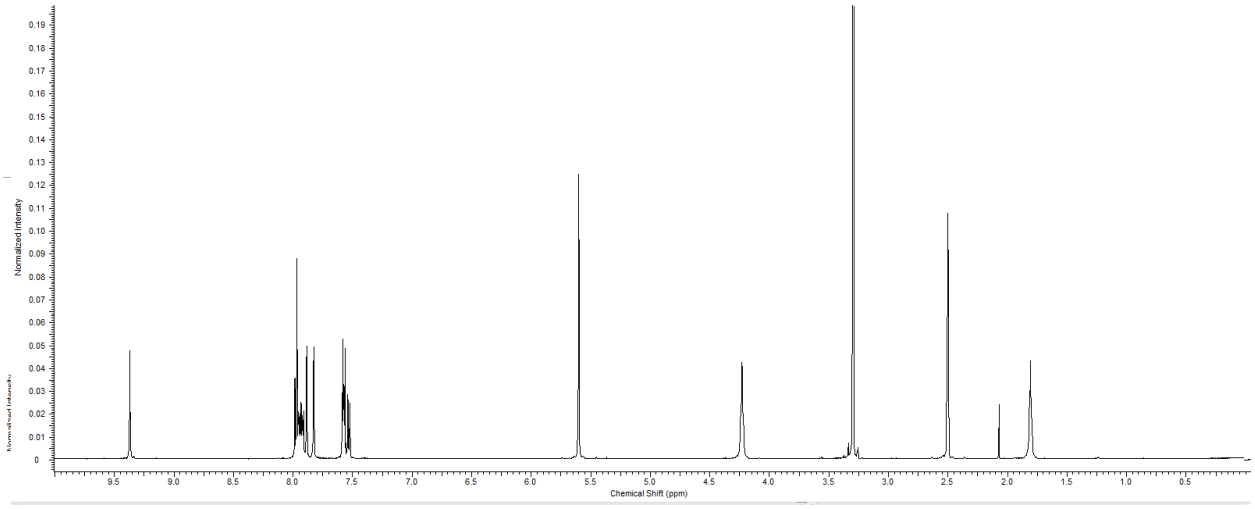


Figure A2.7. ^{1}H NMR spectrum of **4**.

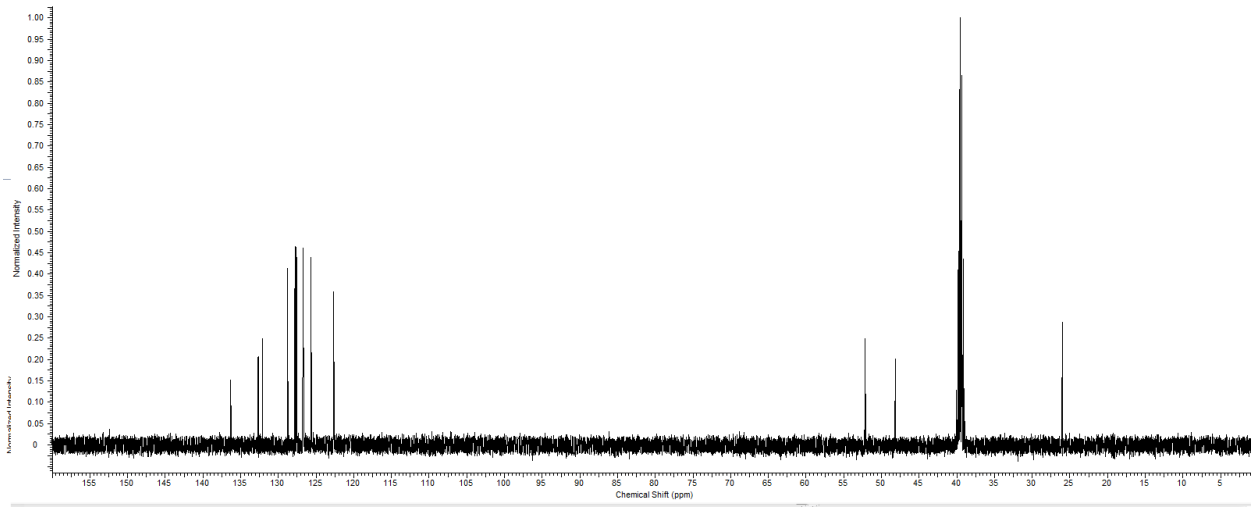


Figure A2.8. ^{13}C NMR spectrum of **4**.

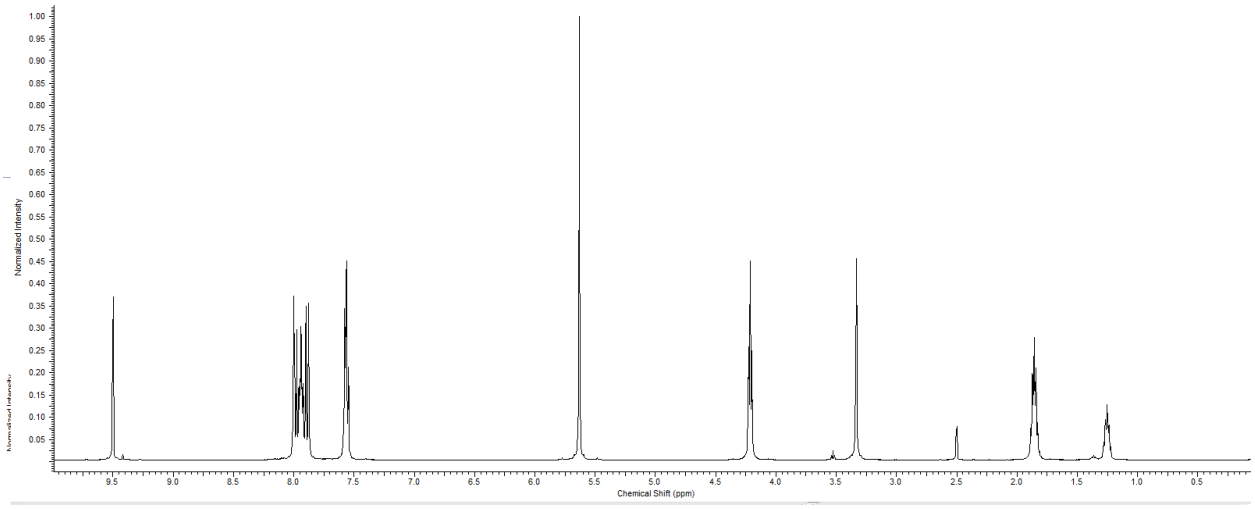


Figure A2.9. ^{1}H NMR spectrum of **5**.

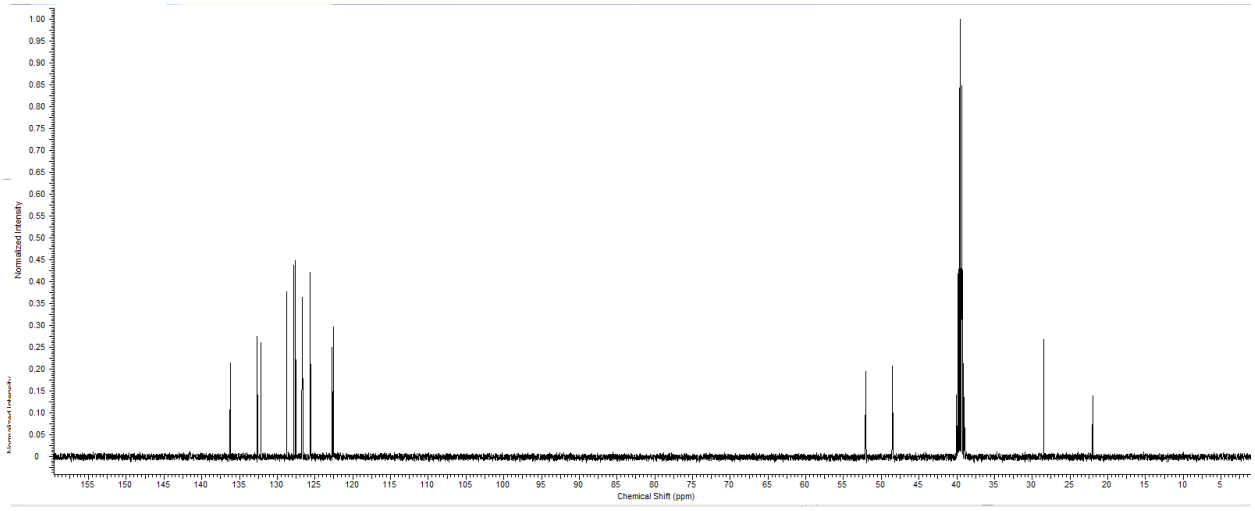


Figure A2.10. ^{13}C NMR spectrum of **5**.

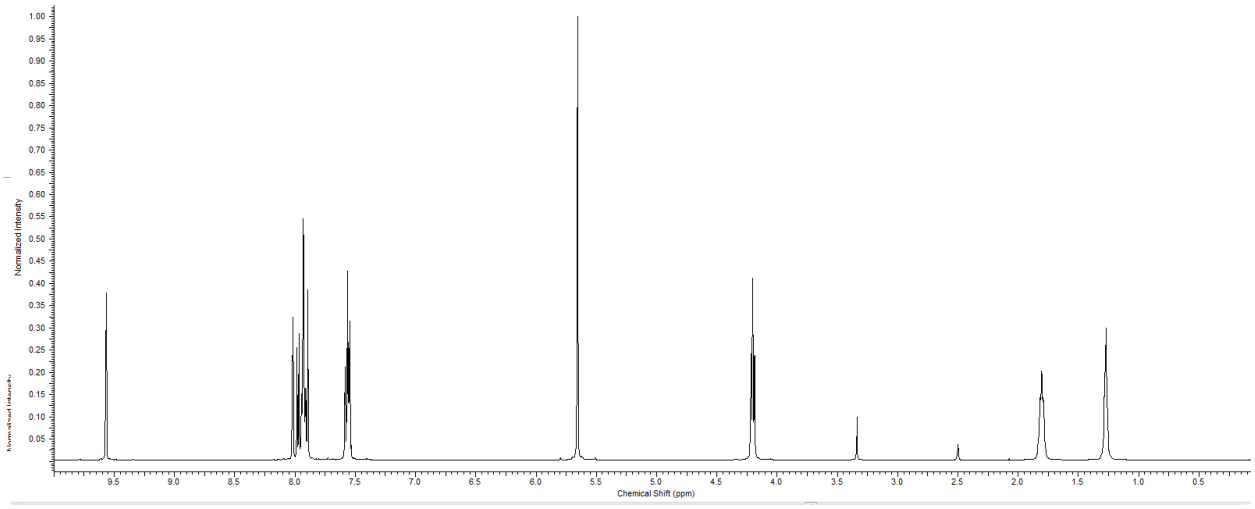


Figure A2.11. ^{1}H NMR spectrum of **6**.

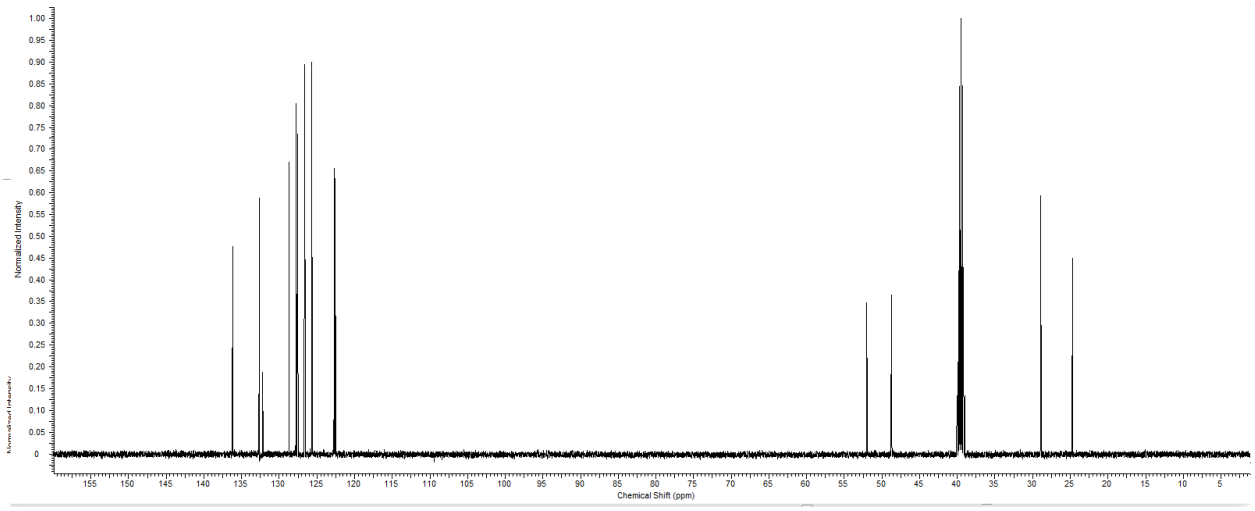


Figure A2.12. ^{13}C NMR spectrum of **6**.

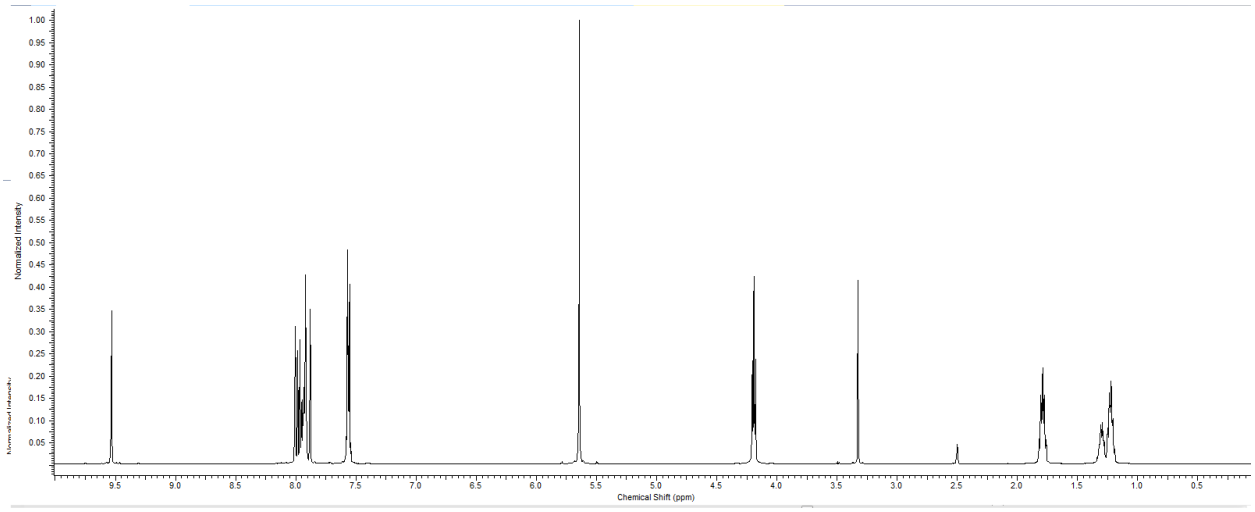


Figure A2.13. ^{1}H NMR spectrum of **7**.

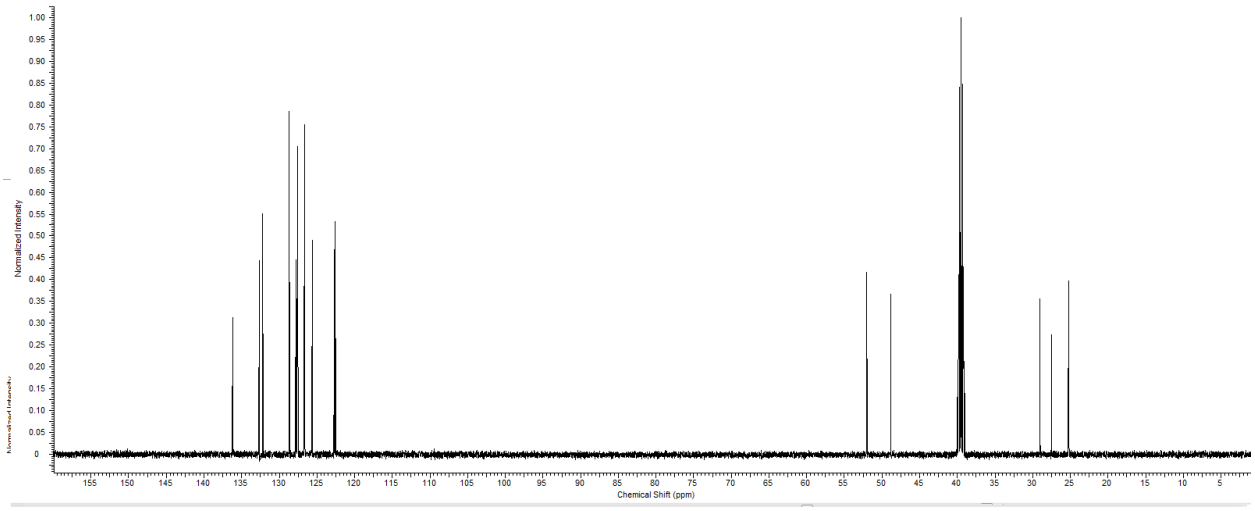


Figure A2.14. ^{13}C NMR spectrum of **7**.

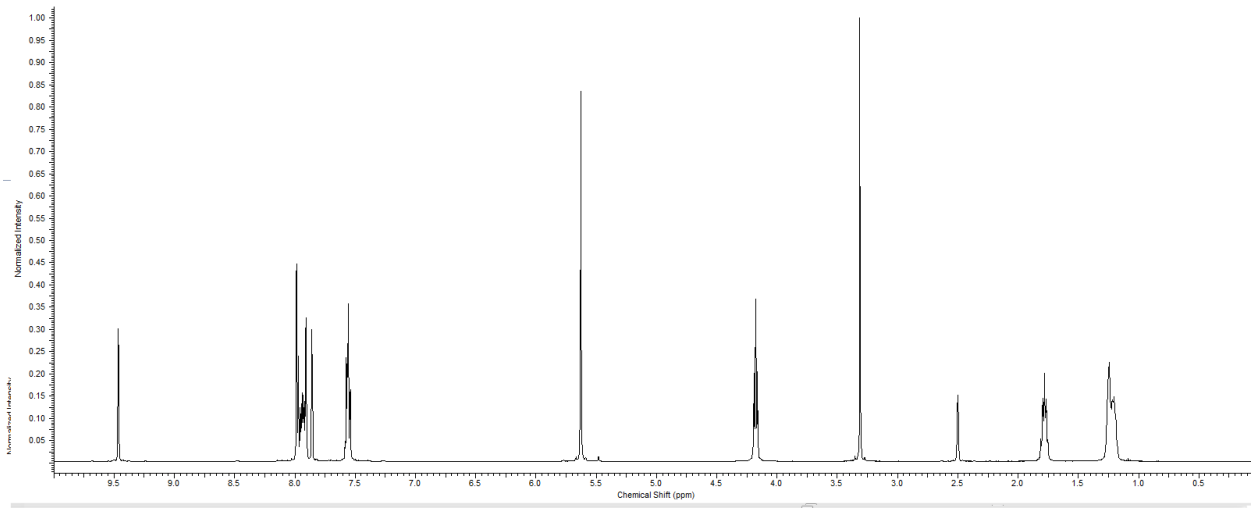


Figure A2.15. ^{1}H NMR spectrum of **8**.

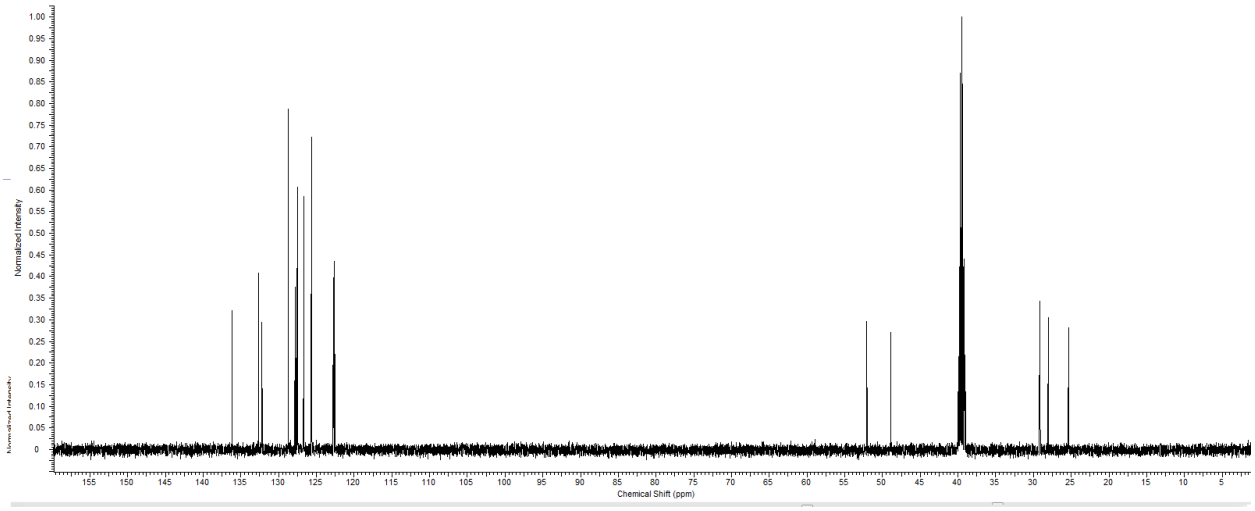


Figure A2.16. ^{13}C NMR spectrum of **8**.

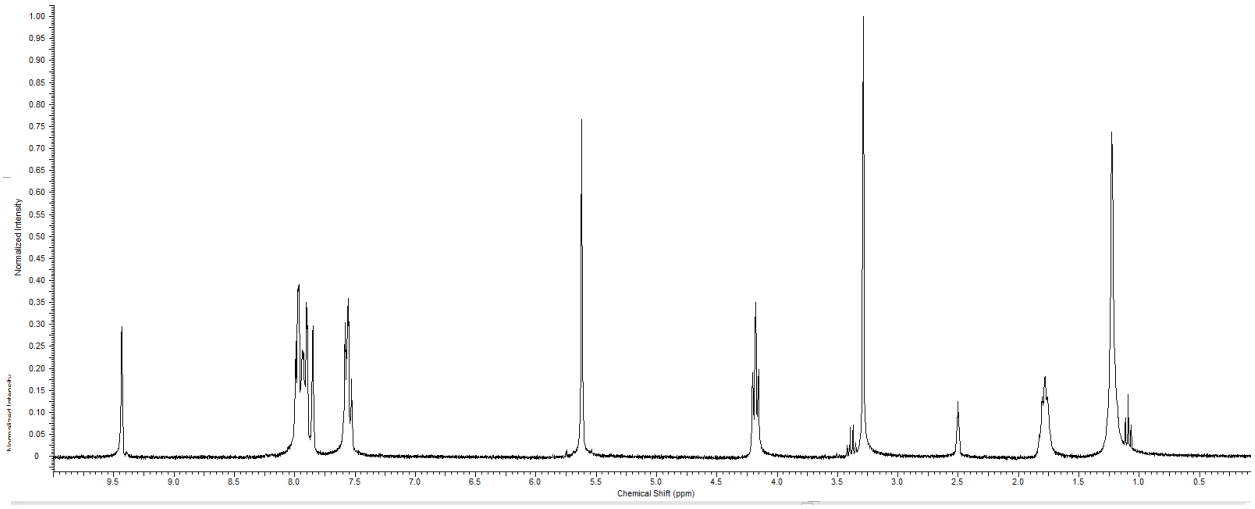


Figure A2.17. ^{1}H NMR spectrum of **9**.

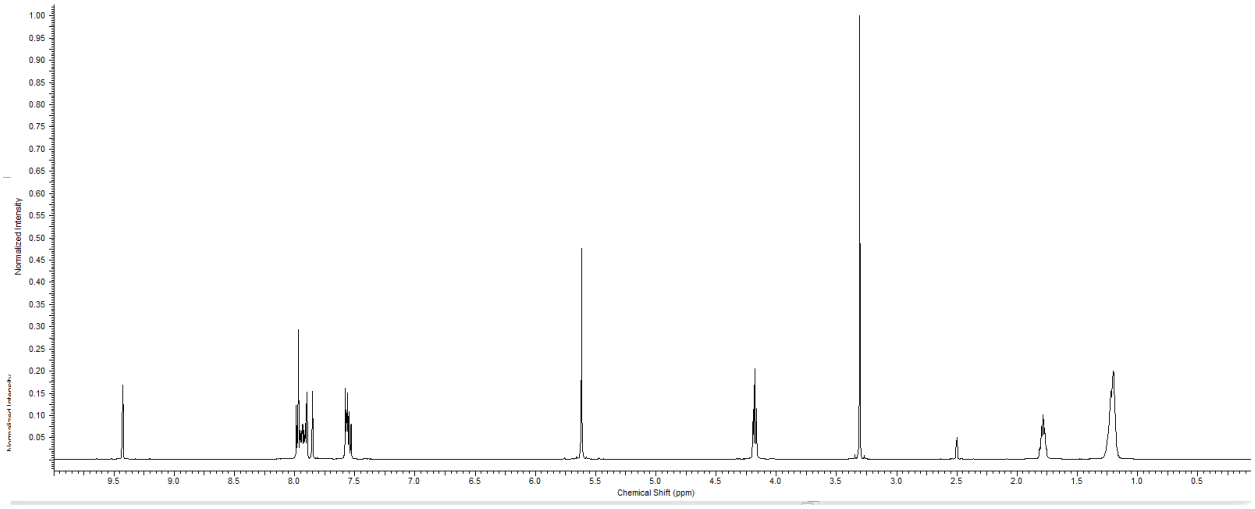


Figure A2.18. ^{1}H NMR spectrum of **10**.

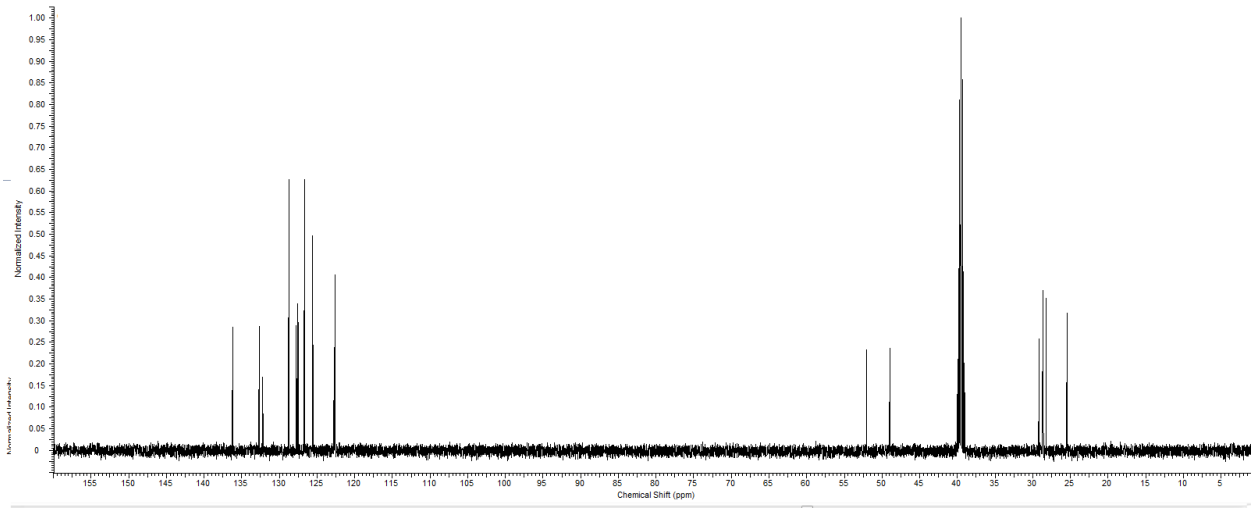


Figure A2.19. ^{13}C NMR spectrum of **10**.

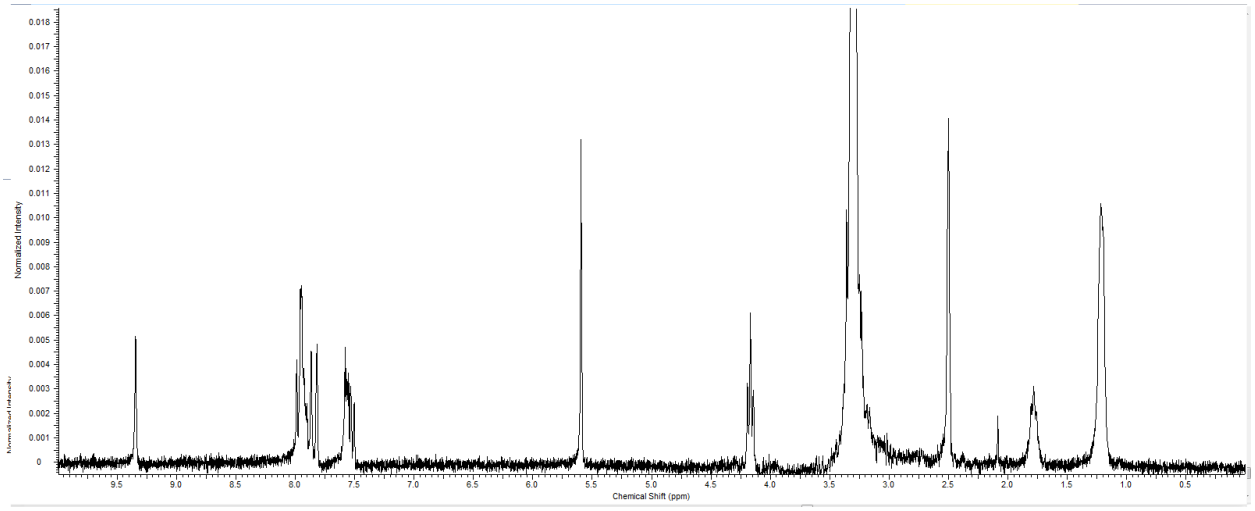


Figure A2.20. ^{1}H NMR spectrum of **11**.

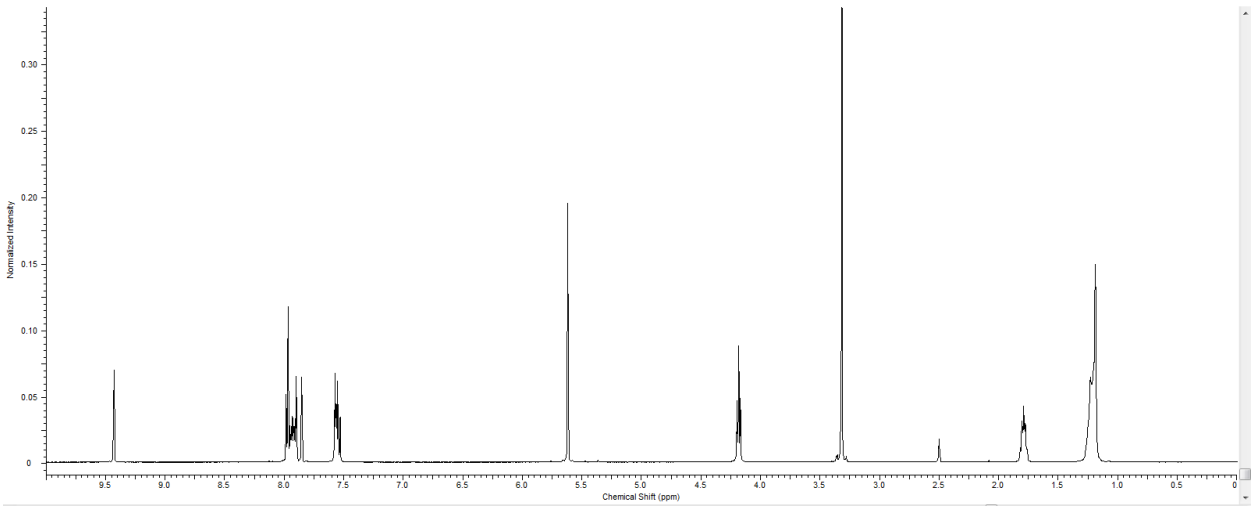


Figure A2.21. ^{1}H NMR spectrum of **12**.

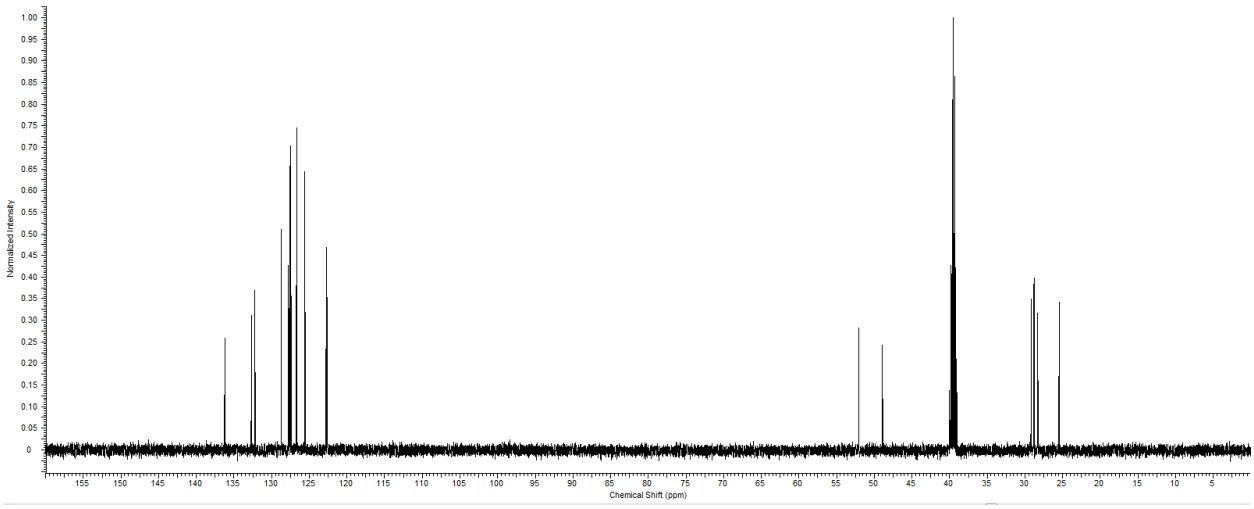


Figure A2.22. ^{13}C NMR spectrum of 1**2**.

Appendix 3: NCI 60 Cell Line Screen Data

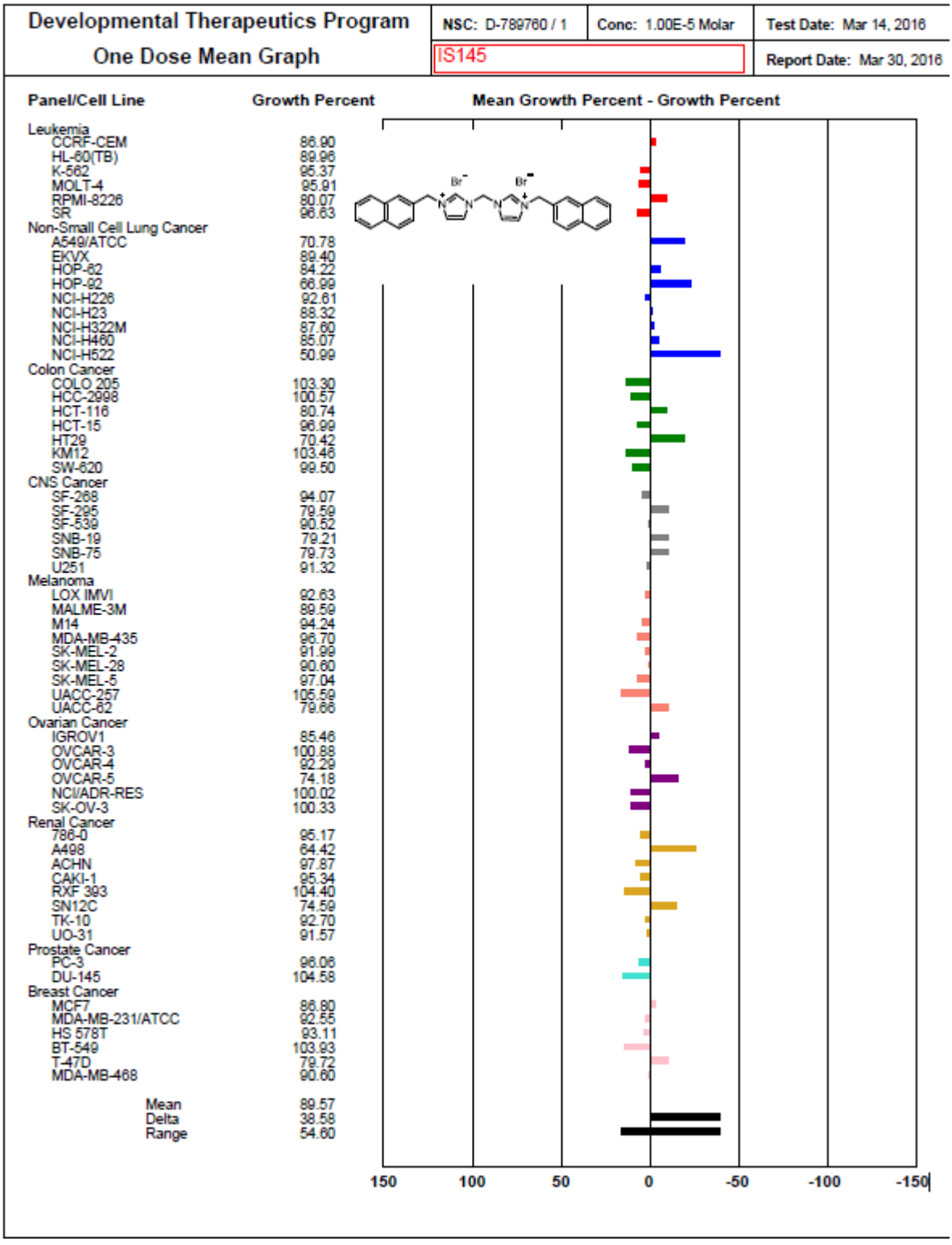


Figure A3.1. 60 cell line screen 1-dose assay for **1**.

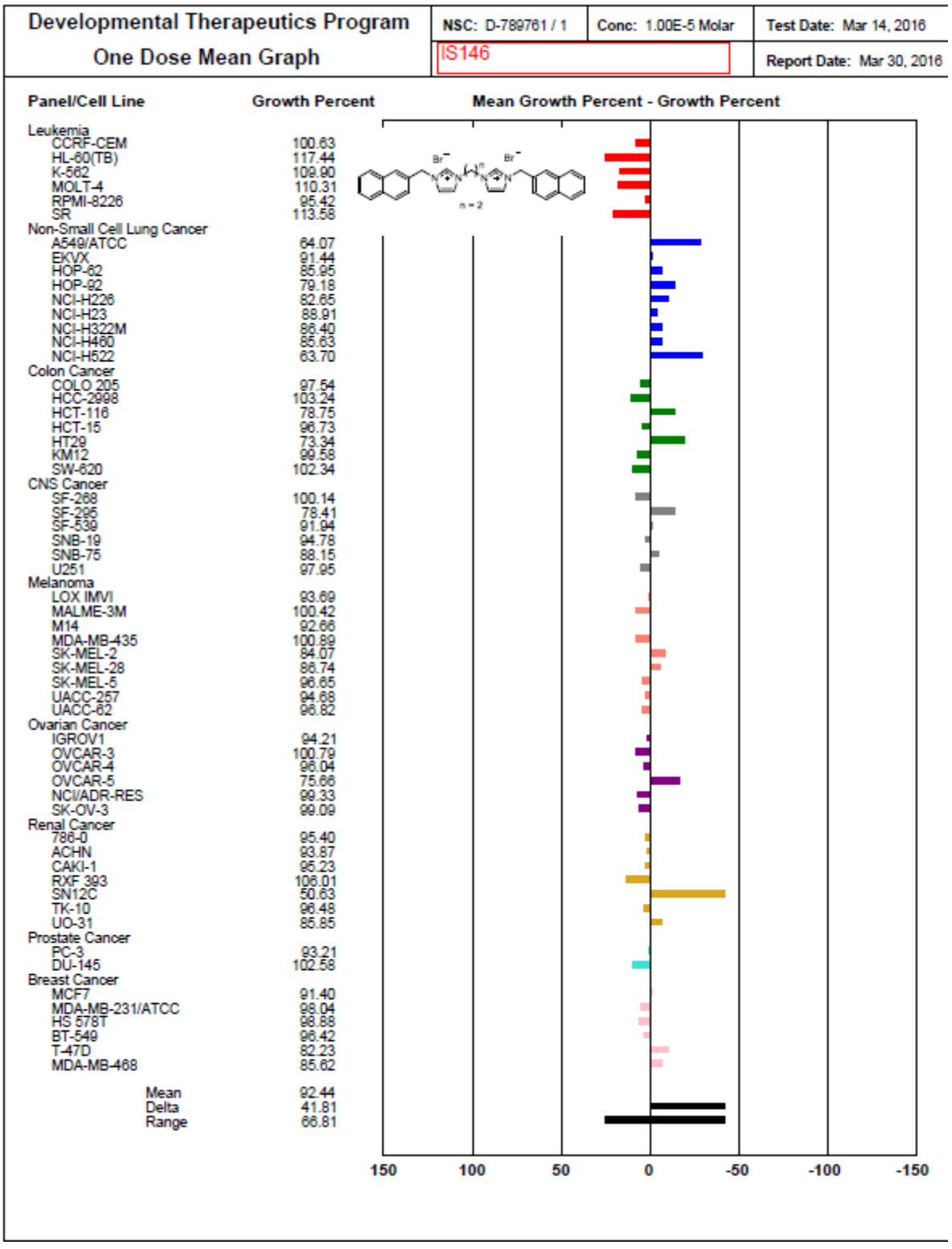


Figure A3.2. 60 cell line screen 1-dose assay for **2**.

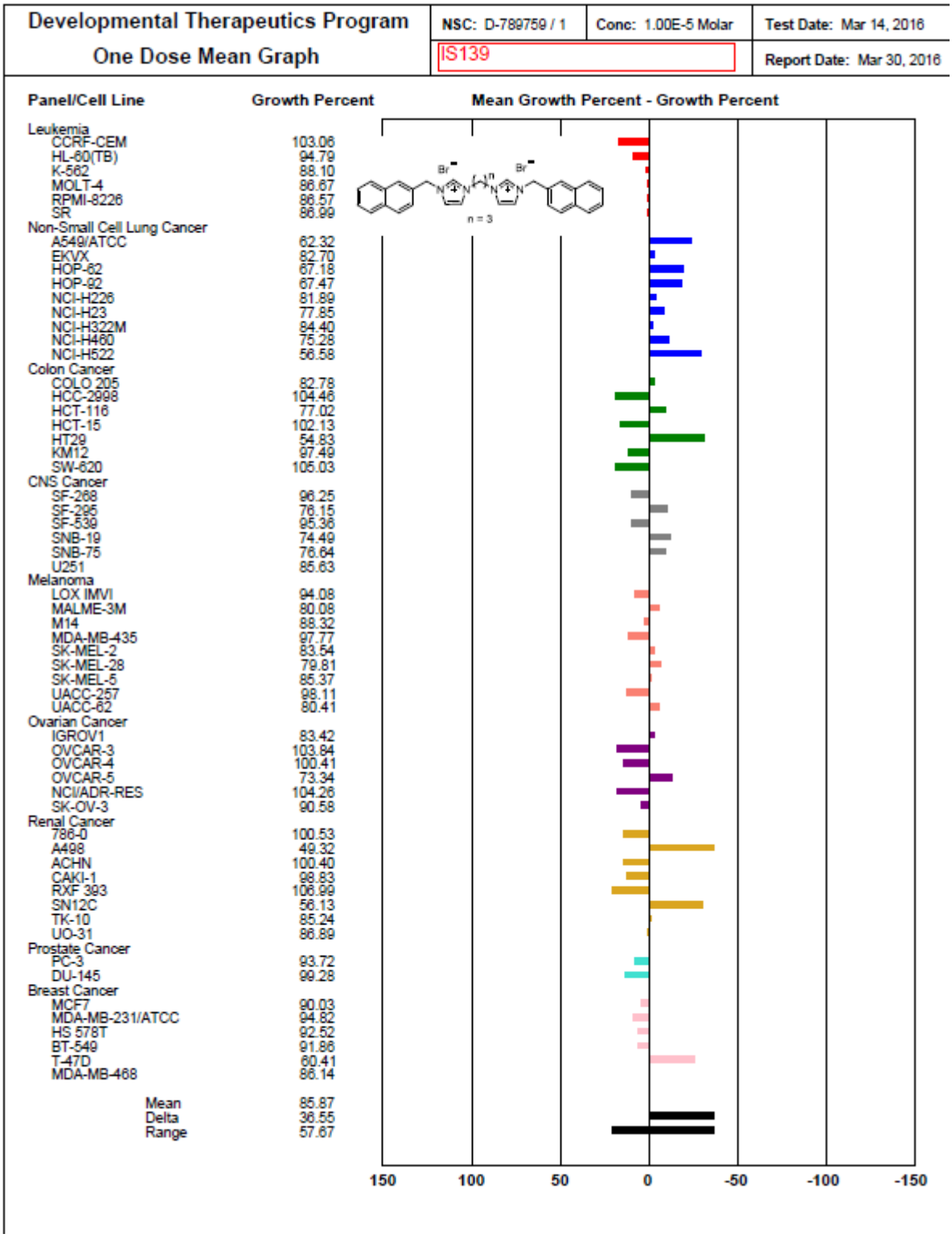


Figure A3.3. 60 cell line screen 1-dose assay for **3**.

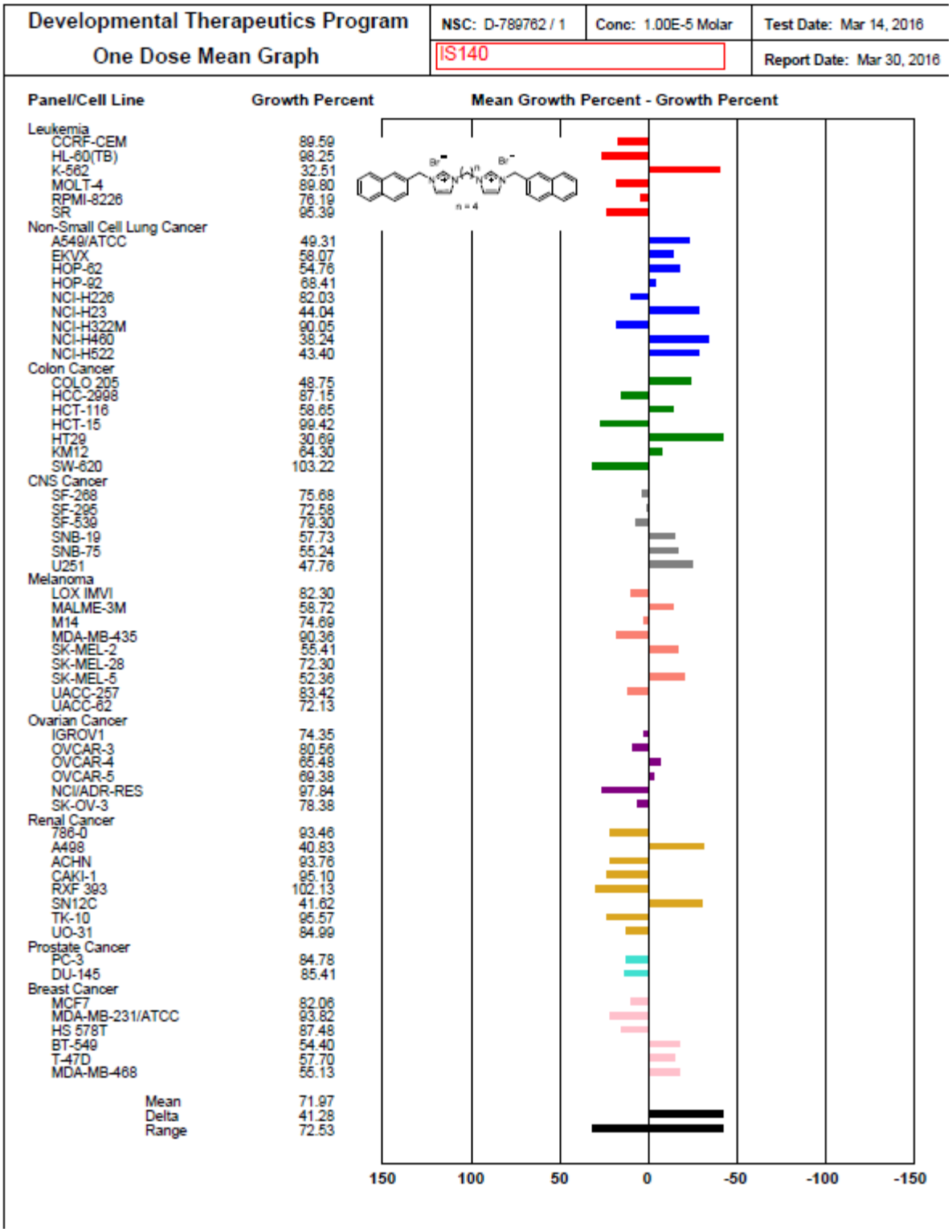


Figure A3.4. 60 cell line screen 1-dose assay for **4**.

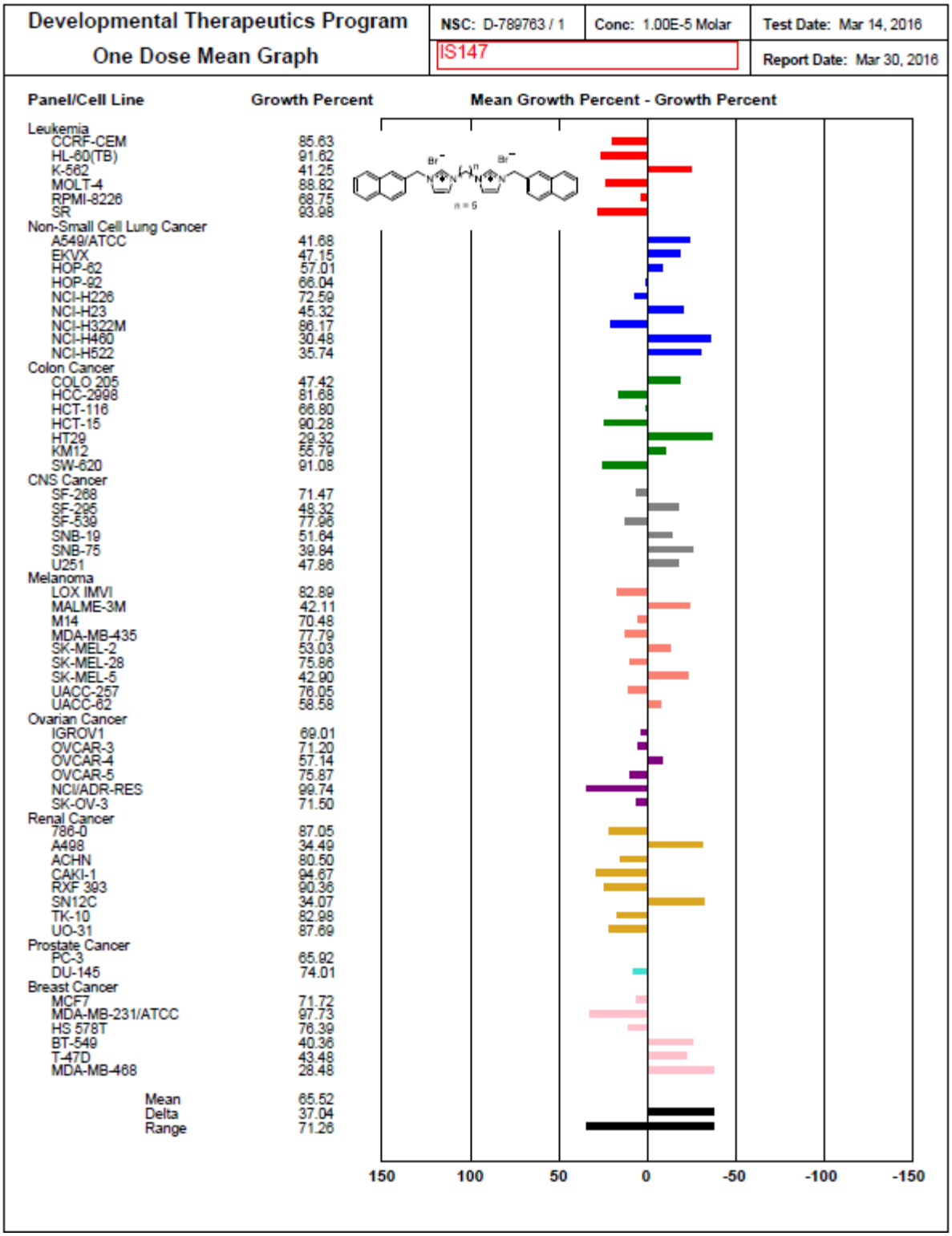


Figure A3.5. 60 cell line screen 1-dose assay for **5**.

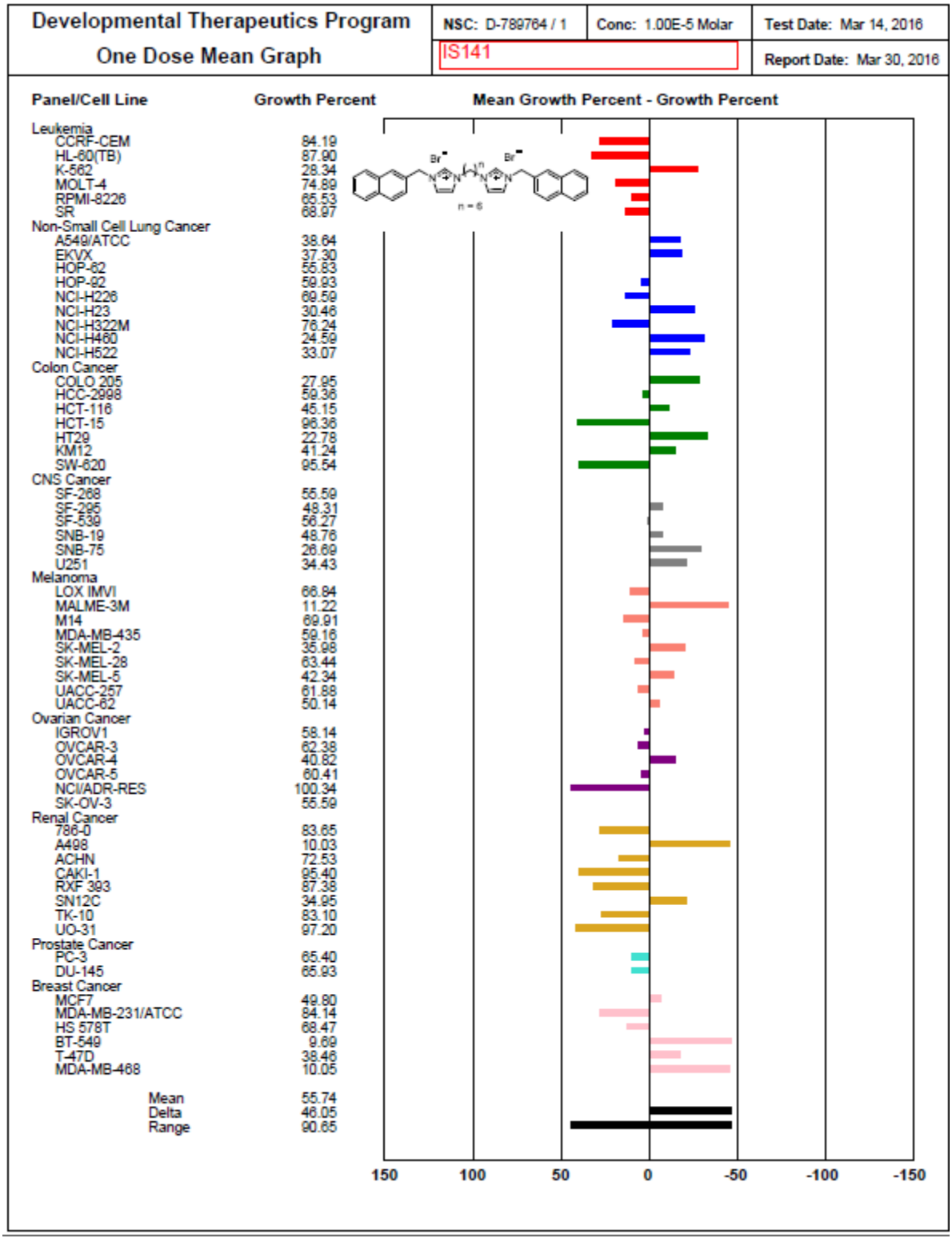


Figure A3.6. 60 cell line screen 1-dose assay for **6**.

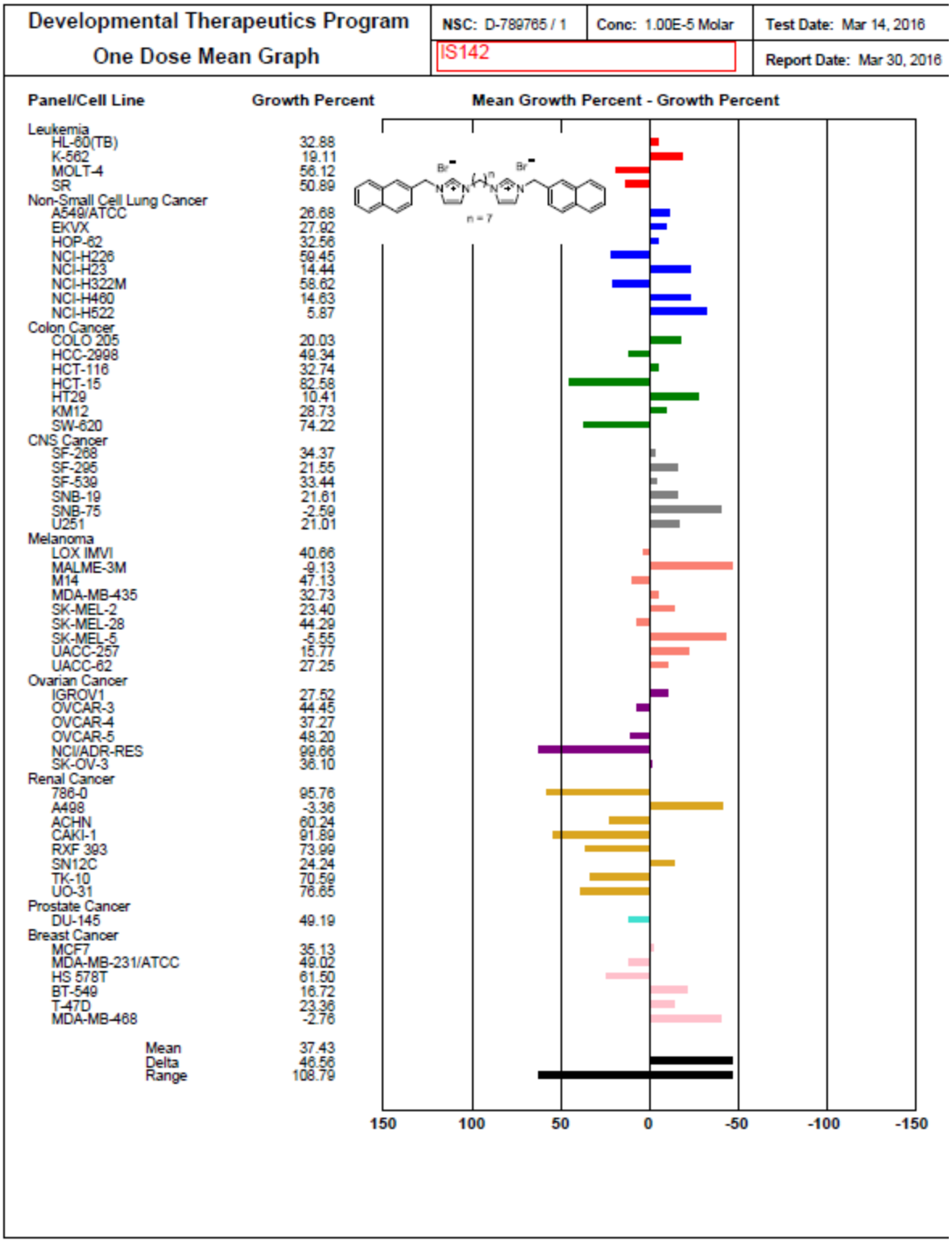


Figure A3.7. 60 cell line screen 1-dose assay for **7**.

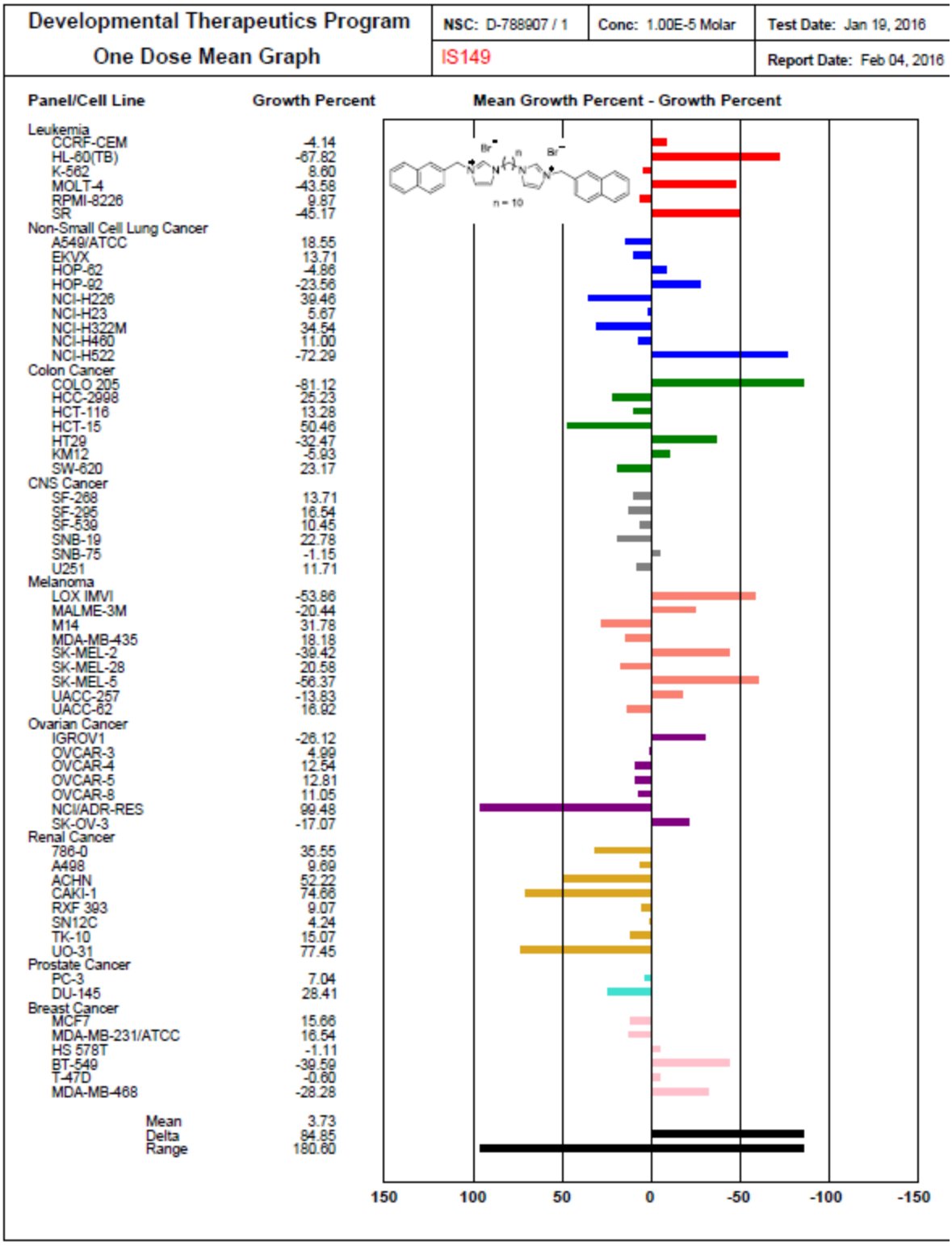


Figure A3.8. 60 cell line screen 1-dose assay for **10**.

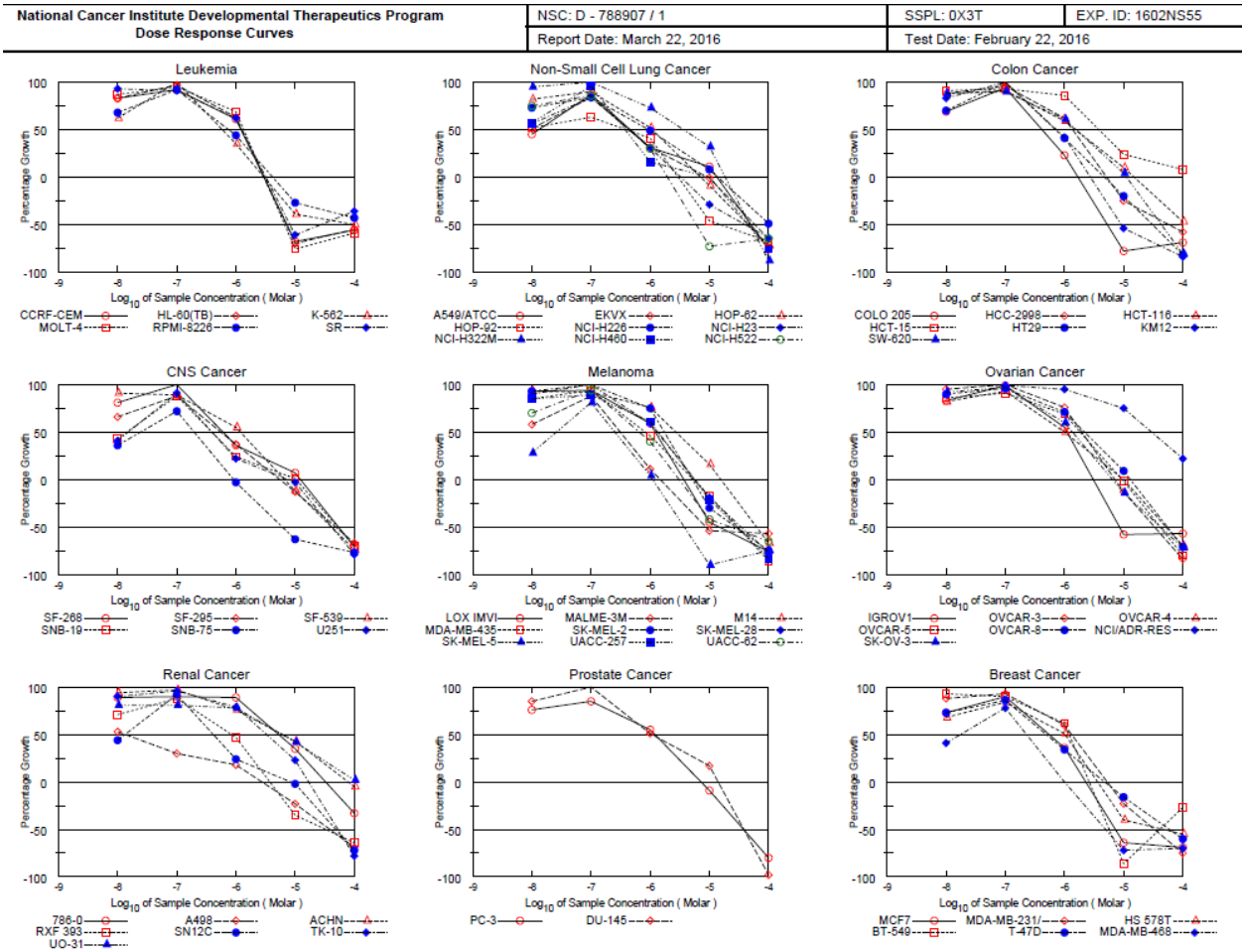


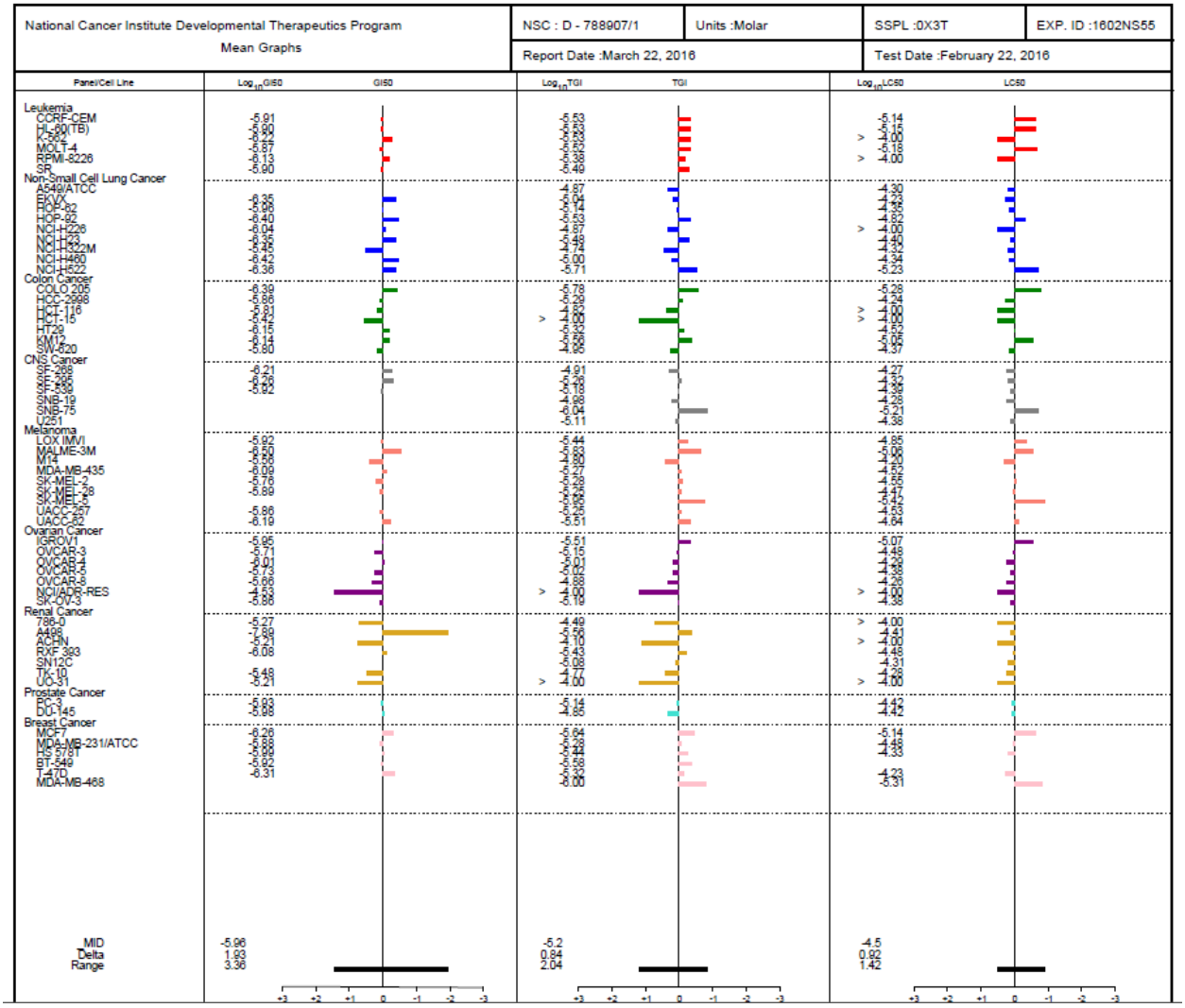
Figure A3.9. 60 cell line screen 5-dose assay dose response curves for **10**.

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 788907 / 1	Experiment ID : 1602NS55	Test Type : 08	Units : Molar
Report Date : March 22, 2016	Test Date : February 22, 2016	QNS :	MC :
COMI : Decyl Bis Cation Nap	Stain Reagent : SRB Dual-Pass Related	SSPL : 0X3T	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.570	2.438	2.129	2.281	1.711	0.182	0.253	83	92	61	-68	-56	1.22E-6	2.97E-6	7.25E-6
HL-60(TB)	0.799	2.363	2.094	2.315	1.786	0.237	0.362	83	97	63	-70	-55	1.25E-6	2.97E-6	7.04E-6
K-562	0.348	1.986	1.361	2.036	0.922	0.212	0.175	62	103	35	-39	-50	6.02E-7	2.97E-6	> 1.00E-4
MOLT-4	0.712	2.650	2.391	2.544	2.052	0.172	0.289	87	95	69	-76	-59	1.36E-6	3.00E-6	6.63E-6
RPMI-8226	0.772	2.640	2.037	2.492	1.586	0.566	0.443	68	92	44	-27	-43	7.37E-7	4.17E-6	> 1.00E-4
SR	0.538	1.759	1.668	1.655	1.293	0.213	0.346	93	91	62	-61	-36	1.25E-6	3.20E-6	.
Non-Small Cell Lung Cancer															
A549/ATCC	0.430	1.964	1.126	1.762	0.910	0.597	0.104	45	87	31	11	-76	.	1.33E-5	5.03E-5
EKVX	0.756	2.476	1.633	2.196	1.298	0.746	0.266	51	84	31	-1	-65	4.42E-7	9.07E-6	5.84E-5
HOP-62	0.608	1.400	1.262	1.318	1.023	0.556	0.168	82	90	52	-9	-72	1.09E-6	7.24E-6	4.45E-5
HOP-92	1.682	2.277	1.989	2.059	1.926	0.908	0.541	52	63	41	-46	-68	3.96E-7	2.96E-6	1.52E-5
NCI-H226	0.901	1.855	1.597	1.702	1.366	0.974	0.457	73	84	49	8	-49	9.18E-7	1.36E-5	> 1.00E-4
NCI-H23	0.746	2.412	1.677	2.148	1.271	0.533	0.267	56	84	31	-29	-64	4.45E-7	3.35E-6	3.99E-5
NCI-H322M	0.771	2.140	2.078	2.147	1.765	1.204	0.093	95	100	73	32	-88	3.55E-6	1.84E-5	4.81E-5
NCI-H460	0.342	3.024	1.875	2.905	0.781	0.352	0.083	57	96	16	.	-76	3.76E-7	1.01E-5	4.59E-5
NCI-H522	0.979	2.043	1.774	1.895	1.294	0.263	0.346	75	86	30	-73	-65	4.36E-7	1.94E-6	5.95E-6
Colon Cancer															
COLO 205	0.476	1.738	1.343	1.658	0.761	0.105	0.146	69	94	23	-78	-69	4.11E-7	1.68E-6	5.26E-6
HCC-2998	0.862	2.895	2.649	2.821	2.123	0.644	0.364	88	96	62	-25	-58	1.37E-6	5.13E-6	5.76E-5
HCT-116	0.405	2.907	2.608	2.679	1.889	0.662	0.214	88	91	59	10	-47	1.55E-6	1.51E-5	> 1.00E-4
HCT-15	0.275	2.188	2.022	2.055	1.911	0.729	0.422	91	93	86	24	8	3.76E-6	> 1.00E-4	> 1.00E-4
HT29	0.301	1.555	1.178	1.578	0.818	0.242	0.053	70	102	41	-20	-82	7.15E-7	4.74E-6	3.04E-5
KM12	0.802	3.236	2.810	3.240	1.823	0.366	0.128	83	100	42	-54	-84	7.27E-7	2.73E-6	9.01E-6
SW-620	0.250	1.617	1.480	1.480	1.086	0.306	0.047	88	90	61	4	-81	1.57E-6	1.12E-5	4.31E-5
CNS Cancer															
SF-268	0.624	2.059	1.791	2.059	1.146	0.725	0.182	81	100	36	7	-71	6.10E-7	1.23E-5	5.40E-5
SF-295	0.753	2.561	1.951	2.348	1.419	0.654	0.247	66	88	37	-13	-67	5.54E-7	5.44E-6	4.79E-5
SF-539	0.891	2.792	2.621	2.576	1.941	0.780	0.230	91	89	55	-12	-74	1.19E-6	6.55E-6	4.06E-5
SNB-19	0.733	2.089	1.316	1.927	1.056	0.753	0.223	43	88	24	1	-70	.	1.05E-5	5.29E-5
SNB-75	1.016	1.732	1.271	1.533	0.983	0.379	0.237	36	72	-3	-63	-77	.	9.06E-7	6.11E-6
U251	0.440	1.901	1.033	1.776	0.765	0.429	0.095	41	91	22	-3	-79	.	7.85E-6	4.21E-5
Melanoma															
LOX IMVI	0.363	2.683	2.527	2.546	1.722	0.198	0.086	93	94	59	-45	-76	1.21E-6	3.66E-6	1.40E-5
MALME-3M	0.808	1.272	1.076	1.217	0.859	0.373	0.351	58	88	11	-54	-57	3.13E-7	1.48E-6	8.71E-6
M14	0.445	1.894	1.796	1.773	1.553	0.683	0.147	93	92	76	16	-67	2.76E-6	1.57E-5	6.24E-5
MDA-MB-435	0.540	2.734	2.397	2.593	1.546	0.451	0.074	85	94	46	-17	-86	8.19E-7	5.43E-6	3.02E-5
SK-MEL-2	1.151	2.207	2.134	2.253	1.938	0.811	0.289	93	104	75	-30	-75	1.72E-6	5.20E-6	2.83E-5
SK-MEL-28	0.924	2.058	1.948	2.087	1.592	0.746	0.215	90	103	59	-19	-77	1.30E-6	5.66E-6	3.42E-5
SK-MEL-5	0.624	2.257	1.080	1.940	0.695	0.066	0.158	28	81	4	-90	-75	.	1.11E-6	3.79E-6
UACC-257	0.877	1.895	1.743	1.786	1.499	0.696	0.153	85	89	61	-21	-83	1.37E-6	5.58E-6	2.98E-5
UACC-62	0.864	2.599	2.087	2.498	1.552	0.505	0.301	70	94	40	-42	-65	6.46E-7	3.08E-6	2.27E-5
Ovarian Cancer															
IGROV1	0.723	2.419	2.133	2.402	1.659	0.305	0.315	83	99	55	-58	-57	1.11E-6	3.08E-6	8.53E-6
OVCAR-3	0.536	1.867	1.802	1.936	1.546	0.465	0.085	95	105	76	-13	-84	1.95E-6	7.09E-6	3.29E-5
OVCAR-4	0.755	1.614	1.463	1.551	1.183	0.753	0.226	82	93	50	.	-70	9.88E-7	9.88E-6	5.15E-5
OVCAR-5	0.819	1.617	1.508	1.542	1.372	0.806	0.163	86	91	69	-2	-80	1.87E-6	9.48E-6	4.13E-5
OVCAR-8	0.633	2.441	2.263	2.371	1.913	0.803	0.185	90	96	71	9	-71	2.18E-6	1.31E-5	5.51E-5
NCI/ADR-RES	0.628	2.232	2.266	2.222	2.157	1.827	0.981	102	99	95	75	22	2.94E-5	> 1.00E-4	> 1.00E-4
SK-OV-3	0.925	2.003	1.918	1.972	1.573	0.791	0.262	92	97	60	-14	-72	1.36E-6	6.39E-6	4.17E-5
Renal Cancer															
786-0	0.754	2.563	2.356	2.378	2.373	1.390	0.503	89	90	89	35	-33	5.33E-6	3.26E-5	> 1.00E-4
A498	1.672	2.170	1.934	1.819	1.762	1.292	0.515	53	30	18	-23	-69	1.29E-8	2.77E-6	3.86E-5
ACHN	0.430	1.803	1.723	1.763	1.477	1.021	0.411	94	97	76	43	-5	6.17E-6	8.03E-5	> 1.00E-4
RXF 393	1.082	1.592	1.444	1.528	1.319	0.704	0.388	71	88	47	-35	-64	8.22E-7	3.72E-6	3.27E-5
SN12C	0.934	2.956	1.819	2.804	1.418	0.914	0.265	44	92	24	-2	-72	.	8.28E-6	4.88E-5
TK-10	1.163	2.519	2.387	2.471	2.229	1.477	0.257	90	96	79	23	-78	3.28E-6	1.69E-5	5.29E-5
UO-31	0.856	2.042	1.821	1.818	1.784	1.359	0.881	81	81	78	42	2	6.15E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.744	2.581	2.135	2.306	1.749	0.680	0.146	76	85	55	-9	-80	1.19E-6	7.30E-6	3.77E-5
DU-145	0.428	1.782	1.585	1.818	1.116	0.663	0.007	85	103	51	17	-98	1.06E-6	1.41E-5	3.81E-5
Breast Cancer															
MCF7	0.344	2.225	1.711	2.032	1.028	0.123	0.105	73	90	36	-64	-69	5.54E-7	2.30E-6	7.22E-6
MDA-MB-231/ATCC	0.707	1.545	1.444	1.483	1.212	0.545	0.175	88	93	60	-23	-75	1.33E-6	5.30E-6	3.29E-5
HS 578T	1.023	1.847	1.586	1.717	1.443	0.613	0.462	68	84	51	-40	-55	1.03E-6	3.63E-6	4.68E-5
BT-549	1.302	2.607	2.511	2.478	2.114	0.179	0.946	93	90	62	-86	-27	1.21E-6	2.62E-6	.
T-47D	0.733	1.246	1.110	1.175	0.907	0.614	0.293	73	86	34	-16	-60	4.91E-7	4.74E-6	5.90E-5
MDA-MB-468	0.666	1.136	0.859	1.031	0.668	0.184	0.198	41	78	.	-72	-70	.	1.01E-6	4.91E-6

‘**Figure A3.10.** 60 cell line screen 5-dose assay in-vitro testing results for **10**.



‘**Figure A3.11.** 60 cell line screen 5-dose assay mean graphs for **10**.

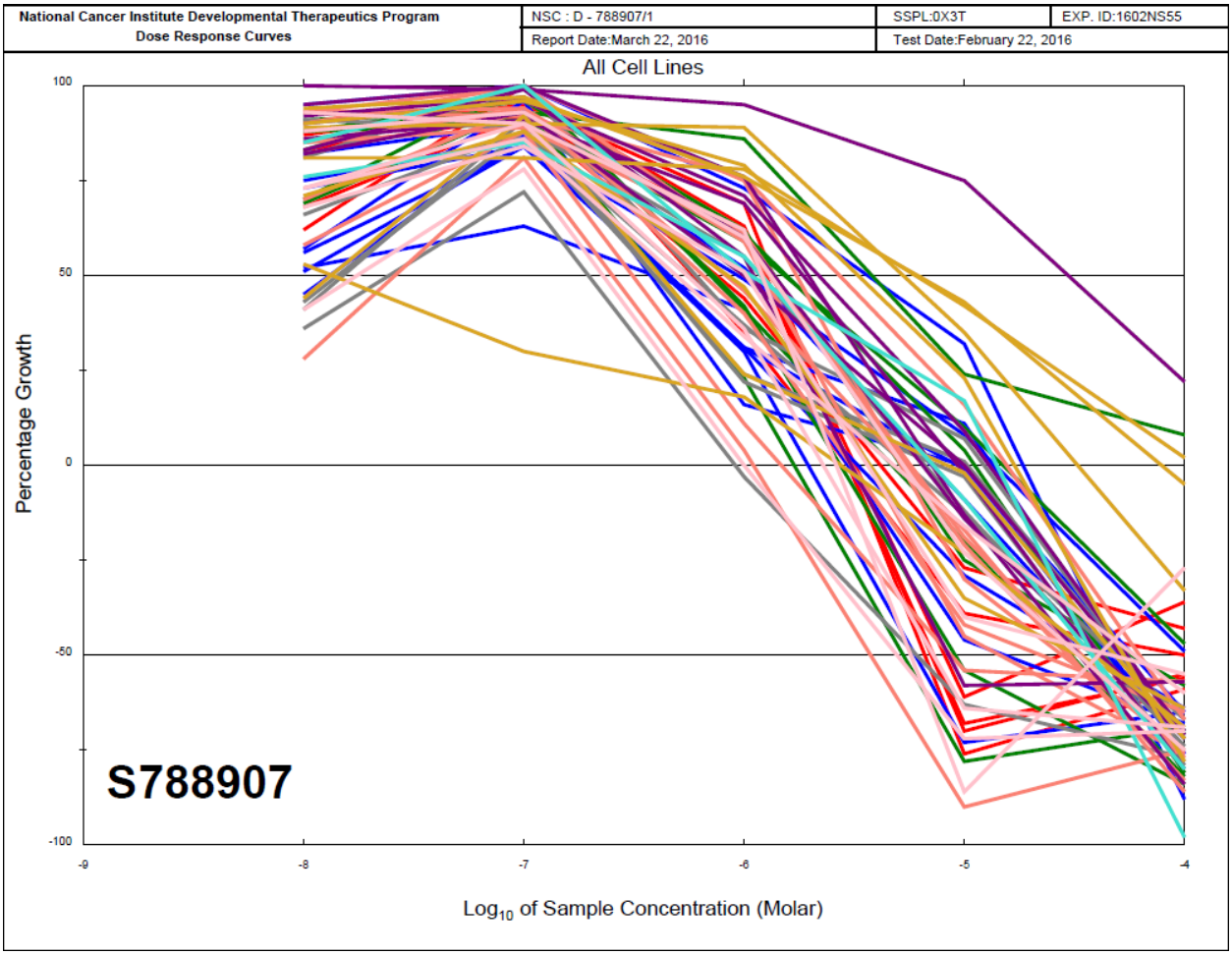


Figure A3.12. 60 cell line screen 5-dose assay dose response curves for all cell lines for **10**.

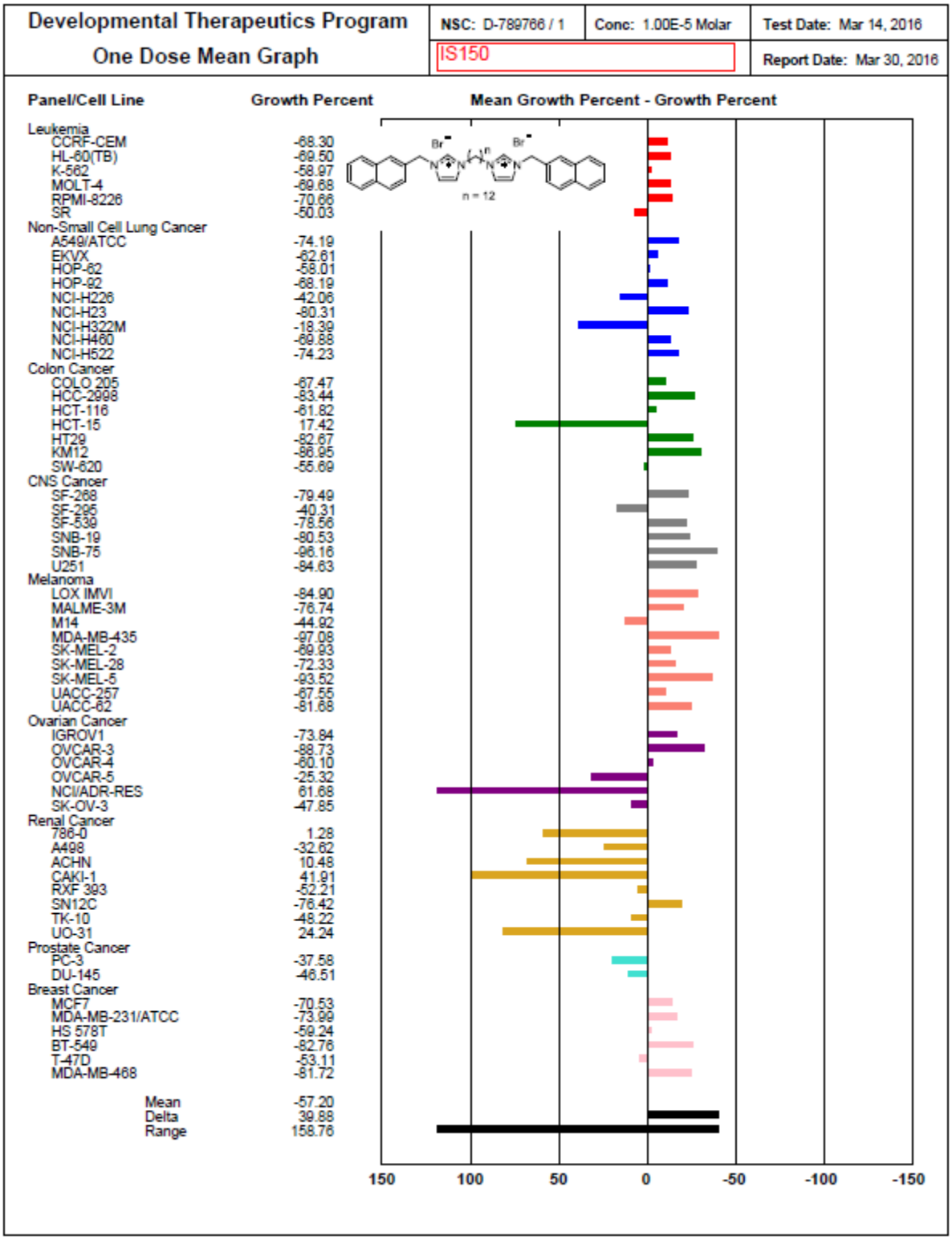


Figure A3.13. 60 cell line screen 1-dose assay for **12**.